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JETHRO TULL

IN MEMORIAM

CHARLES A. SHULL

(WITH ONE PLATE)

The two-hundredth anniversary of the death of JETHRO TULL occurred in the early days of March, 1940, the exact date being unknown; he was buried on March 9, 1740, at Basildon, Berkshire, England, amid such quiet and lack of publicity that no public record of his death and burial place was made. This anniversary should not be allowed to pass by without refreshing our memories of the man, who, more than any one else, was responsible for the development of genuine cultivation of the soil to improve its fertility. A celebration of the 200th anniversary of the publication of his book was held at the 10th annual meeting of the American Society of Plant Physiologists in 1933. The writer's address on that occasion was not published because it was an illustrated, informal discussion. This memorial presents the main facts of TULL's life as brought out in the address before the Boston meeting.

JETHRO TULL stands out as a great leader in an age of ignorance concerning the productiveness of the earth. As a contemporary of STEPHEN HALES, he shares the honor of leading in the development of new ideas. HALES was the more scientific, TULL the more practical; one foreshadowed the development of the fundamentals of plant physiology, the other the fundamentals of practical utilization of soils and crops for human welfare. Both deserve a full measure of praise from present day scientists whose work continues, deepens, and strengthens the principles discovered by these pioneers.

There were other writers in the agricultural field before JETHRO TULL, of course. The following are a few of them: FITZHERBERT, author of *The Boke of Husbandrie*, in 1534; RICHARD WESTON, with his *Discourse of Husbandry as Used in Flanders*, 1645; JOHN EVELYN'S *Terra*, 1658; and

JOHN MORTIMER's *Whole Art of Husbandry*, 1707. These are the better known observers and recorders previous to TULL's time.

It was JETHRO TULL's belief in the soil, his originality and independent thinking, his keen observations, his intuitive perception of causes and effects, and his courageous industry in the face of great trials and difficulties that made him a great agricultural leader during the first four decades of the eighteenth century. It was undoubtedly accidents of life, inheritance or acquisition of property, that led him away from legal, political, and cultural pursuits, and to a life of struggle with the forces of nature, and to a struggle against human inertia, stubborn ignorance of the common people, and the persecutions of his detractors and defamers. The resistance of laborers against his innovations in practice, and his ingenious inventions, which required them to perform unwonted tasks, is a revealing commentary on the times.

These laborers were insolent, disobedient, and hated the new practices and the new tools. The tools were often broken surreptitiously by some sullen workman, to make them look impractical; or parts were thrown into the well so that they need not be used. Careful directions for seeding and culture were obstinately disobeyed; or plants deliberately destroyed to prevent success with the experiments. If you wonder why he didn't fire such workmen, as we would today, it is explained by the laws which were designed to protect these ignorant laborers from injustice. The landlord was practically owned and run by the workers, who could not easily be dispossessed of their means of livelihood for their malpractices.

Many nasty epithets were used, to make TULL look ridiculous. After his book appeared his detractors tormented him with scurrilous criticisms. One group of these, a society headed by one STEPHEN SWITZER, had an anonymous writer who called himself "Equivocus." TULL called the whole set "The Equivocal Society." Earl CATHCART quotes TULL's comments on these tormenters as follows: "The Secret Society—the Equivocal Society—likewise are not content with abusing my vegetable principles, and terming me an atheist, but also describe me by a similitude of the most odious, despicable, and pestiferous animals. They also usurp the power of the Inquisition in damning books because not their own. And, again, they, the critics in question, seldom make use of any other logic than that of Billingsgate; they call me names—atheist, infidel, fool, mente captus, madman, ass, owl, viper, carping insect, &c, all feminine arguments of scurrility." Time has put these braying asses where they belong, and TULL's principles are immortalized in all of the arable lands of the earth.

Not only was TULL beset by low-browed workers and critics; he was ill most of his life, with kidney stones, and other equally painful and at that time incurable diseases. He developed a chest ailment for which he had

to spend several years in sunny Italy. In spite of pain and disease his mind was ever at work, thinking up new practices, new instruments, plows, harrows, drills, cultivators, hoes, etc., to give more effective nourishment to crops, more even stands of grain, to use less seed, to secure better germination, and a better, more fertile soil.

His home life must not have been very congenial. His wife was probably not overly sympathetic with her suffering husband; and his only son turned out to be a ne'er-do-well. He had no one to turn to in those moments when he most needed understanding and faithful, human companionship.

It was in the midst of such harassments that his great book, *The Horse Hoeing Husbandry*, was written. He had to depend upon a scribe for the preparation of his manuscript from his notes; and the scribe was not careful enough to get things down in the right order. The publishers, also, were not sufficiently cooperative with the proofs and changes before the work was actually printed. TULL explains all of these difficulties in his preface, and gives a clear picture of the tremendous odds against which he struggled. It was at the urging of many really interested and notable personages, who visited him at Prosperous Farm, and who saw the true value of TULL'S experiments that he at last consented to make a permanent record of his ideas, discoveries, and inventions.

Turning now to his life story, which may be told very briefly, he was the son of JETHRO and DOROTHY TULL. His father, JETHRO, SR., came from Midgham, in the Kennet valley, between Newbury and Reading; and his grandfather, GILES TULL, had been the church warden at Midgham in 1641.

His father and mother moved to Basildon, in Berkshire, and were living there when JETHRO, JR. was born, sometime in March, 1674. The church records show that JETHRO was baptized on March 30, 1674.

Little is known of his boyhood; but when he was 17 years old he went to Oxford, where he matriculated in St. John's College on July 7, 1691. He did not finish the course at Oxford, and a couple years later he was admitted as a law student at Gray's Inn, London, December 11, 1693. He also spent two years at Staple Inn. He had hoped to make his contribution to political life, and was interested mainly in the principles of the English constitution, not in the practice of law.

After his student days had ended, he made a grand tour of the continent of Europe. This was sometime between 1693 and 1699, and he took careful notes on the soils, methods, implements, vegetable productions, and any agricultural practices employed in the lands he visited. The culture of vineyards in France was especially enlightening, and he was struck with the idea of applying similar deep culture to all agricultural crops.

He was admitted to the bar on May 19, 1699, after his return from his continental travels; and in the autumn of that year, October 26, 1699, was

married to SUSANNA SMITH of Burton-Dassett, Warwickshire. There were five children born to them, one son and four daughters.

Their first home was at Howberry, Crowmarsh, just across the Thames from Wallingford in Oxfordshire. Here JETHRO engaged in farming, and invented many of his most useful tools. The drill is believed to have been invented about 1701.

After almost a decade of hard labor at Howberry, he moved to Prosperous Farm in Berkshire. Shortly thereafter he spent three years, 1711 to 1714, in Italy, as has been mentioned. On his return to Prosperous Farm, he made of it a living demonstration of his practical ideals. Many notables visited him, and carried away new hope for agriculture in the British Isles. The new culture was taken up in Scotland, and wherever it was diligently applied, great improvement of agriculture resulted. It was only the importunity of these interested people who saw beyond the horizons of the day that finally induced TULL to take up the painful and distasteful duty of writing his book.

He lived at Prosperous Farm, in the southwestern corner of Berkshire, for 26 years, until his death, in 1740. So little attention was paid to his passing away that no public record was made of his burial place; and for nearly 150 years his last resting place remained unknown. But JETHRO TULL's fame, shining ever more brightly out of the darkness of the early eighteenth century finally challenged intelligent and devoted research to locate his burial place. In 1889, after 20 years of patient search, the problem was solved by WALTER MONEY, who discovered the church record of his burial in the Parish Register of Basildon, which reads: "JETHRO TULL gentleman of the Parish of Shalburne was buried March ye 9th 1740. Mem. This JETHRO TULL Esquire was the author of a valuable book on agriculture, entitled Horse Husbandry. GEO: BELLAS, Rector." GEORGE BELLAS was vicar at Basildon at the time of TULL's death and burial, and rector of the neighboring Parish of Yattenden.

Thus the life of this great man is marked at the beginning and the end, not by the actual dates of birth and death, but of baptism, and burial. In the light of our day, TULL stands out as one of the really great benefactors of mankind, whose principles now form the basis of all intelligent and successful cultivation of the earth, and of man's escape from the gloomy predictions of MALTHUS. The breaking of the soil, the drilling of the seed, the fruitful yields of the fertile earth form a perpetual and enduring monument to his genius. May we never forget how much we owe him.

THE UNIVERSITY OF CHICAGO

INHIBITION OF INCREASE AND ACTIVITY OF TOBACCO-MOSAIC VIRUS UNDER NITROGEN-DEFICIENT CONDITIONS

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(WITH ONE FIGURE)

Introduction

Although viruses cause many plant diseases, little is known regarding their metabolism since they apparently multiply only in living tissue. Here it is difficult to differentiate between the metabolism of a host and that of a pathogen. Multiplication of viruses *in vivo* is a well recognized fact and thermal inactivation *in vivo* of a few viruses has been definitely established. No other method for the inactivation of virus *in vivo* has been demonstrated experimentally.

In this study on virus metabolism, a knowledge of the nitrogen metabolism of normal tobacco plants and the discovery that nitrogen supplied to a virus-infected plant had an effect on virus concentration (**19**) have made it possible to distinguish to some extent between the anabolic and katabolic processes involved. It is well known (**14, 24**) that in mature leaves of a nitrogen-deficient plant proteins are hydrolyzed and proteolytic decomposition products liberated. These are translocated to the growing point where they supply the meristem with materials necessary for protein synthesis. A study of the effect of withholding nitrogen from a tobacco plant diseased with tobacco mosaic on the total quantity and specific biological activity of virus within the plant has thrown light on the following questions: Is the virus a stable entity under conditions of nitrogen deficiency, or is it subject to normal protein hydrolysis with a subsequent loss in either amount or activity or both? If the virus is hydrolyzed, can the proteolytic products then be used in meeting the normal nitrogen requirements of the plant, or can they be reassembled into fully active virus? A series of experiments were carried out in an effort to determine in what way tobacco-mosaic virus is affected in a nitrogen-deficient plant. Such studies have been made possible by a combination of the techniques of growing plants in nutrient culture, purification of virus by high-speed centrifugation, and estimation of virus activity by a modification of the local lesion method. The results of the studies, a partial summary of which has been published (**20**), are reported in this paper.

Experimental procedure

Turkish tobacco plants (*Nicotiana tabacum* L.) were used as host plants in all experiments. Uniform seedlings in the 3- or 4-leaf stage were trans-

planted into 4-inch porous clay pots filled with washed, white quartz sand. The pots were placed in clay saucers on a bench in a greenhouse, the temperature of which was held between 70° and 80° C. most of the time. Three days after being transplanted, the plants were inoculated with fresh juice from a tobacco plant diseased with tobacco-mosaic virus (*Marmor tabaci* H.).[1] Nutrient treatments were started at time of inoculation. Each plant received 100 ml. of a complete nutrient solution every second day and water between nutrient applications whenever necessary. The nitrogen level of the solution (200 p.p.m. of nitrogen) had been found in previous tests to produce normal growth comparable to that obtained in a rich composted greenhouse soil. The composition of the solution, together with that of a solution deficient only in nitrogen, reference to which will be made later, is given in table I. In addition to the salts listed in the table, each solution

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS

NUTRIENT SOLUTIONS	VOLUME OF 0.5 MOLAR STOCK SOLUTIONS PER LITER OF NUTRIENT SOLUTION				
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	$(NH_4)_2SO_4$	$CaCl_2$
	ml.	*ml.*	*ml.*	*ml.*	*ml.*
Complete	12.9	11.0	4.0	3.3	0.0
Minus nitrogen	12.9	0.0	4.0	0.0	11.0

was supplemented with $\frac{1}{2}$ p.p.m. of both boron and manganese as H_3BO_3 and $MnSO_4$, respectively.

At intervals, representative plants were cut, weighed, and then placed in covered pans in a cold room held at −14° C. Twenty-four hours later the frozen plants were minced in a food chopper. The pulp was mixed with 3 per cent. by weight of K_2HPO_4 and allowed to thaw at room temperature. The cold juice was expressed from the pulp through cheese cloth by means of a small screw press and cleared of all extraneous insoluble materials by low-speed centrifugation. Aliquots of this juice were then assayed for virus activity, total protein, and total virus protein. The assay of plant proteins included all soluble protein fractions. No account was taken of the insoluble fractions.

Virus activity of juice from diseased plants was assayed by means of the local lesion method (**4**), using bean (*Phaseolus vulgaris* L. var. Early Golden Cluster) as the test plant (**16**). Although the use of this method does not give a measure of the absolute amount of active virus present, certain modifications have recently been made by means of which it is possible to esti-

[1] Latin names of viruses used in this paper were taken from the Handbook of Phytopathogenic Viruses (5).

mate, usually within 10 to 20 per cent., the quantitative difference in virus activity between two or more samples. Data on this modified method and experiments pertaining to its accuracy will be published later (**22**).

Total soluble protein content of juice was determined by precipitation of the protein by hot trichloroacetic acid. One ml. of juice was treated with 1 ml. of hot 5 per cent. trichloroacetic acid and immediately cooled. Following centrifugation, the denatured protein was dissolved in 1 ml. of 0.2 N NaOH, reprecipitated with 1 ml. of 10 per cent. trichloroacetic acid and again centrifuged. The protein precipitate was transferred to a micro-Kjeldahl flask with 1 ml. of 0.2 N NaOH and analyzed for Kjeldahl nitrogen by the modified method (**11, 15**) of FOLIN and FARMER (**3**), as follows: To the protein solution was added 2 ml. of concentrated H_2SO_4, 1 gm. of K_2SO_4, 2 drops of SeOCl, and a few alundum chips. The mixture was digested for at least 5 minutes after the contents had become clear or straw-colored. After the addition of 30 ml. of water and 6 ml. of 50 per cent. NaOH, nitrogen as ammonia was distilled over into 0.02 N HCl. All titrations were made with 0.02 N NaOH, using methyl red as an indicator. This determination included both soluble plant protein and virus protein.

The virus protein was isolated by means of an air-driven ultracentrifuge as previously described (**21**). Stainless steel centrifuge tubes were used in place of thin-walled celluloid tubes. The content of sedimented virus, as measured by the virus-protein content, was then assayed by digestion of the virus protein with H_2SO_4, K_2SO_4, and SeOCl, as outlined above.

This experiment was carried out during September and October. Similar experiments conducted during November and December and again during early summer gave comparable results.

Results

A study was first made of the effect of nitrogen starvation on young plants showing severe systemic symptoms of tobacco mosaic. Starting at time of inoculation, all plants were fed the complete nutrient solution. Ten days after inoculation the plants were divided into two groups. One group continued to receive the complete nutrient solution every second day. The other group, after a thorough flushing of the sand to remove as much nitrogen as possible, was fed the minus-nitrogen solution every second day. At 4-day intervals, representative plants from each group were harvested and analyzed for total protein, virus protein, and virus activity.

In table II are presented data pertaining to the growth of diseased plants that received the two nutrient treatments. Four days after transfer, the nitrogen-fed plants weighed about 15 per cent. more than those deprived of nitrogen. With each succeeding harvest thereafter, the difference in size of plants between the two groups became progressively greater, until by the

24th day the nitrogen-fed plants were nearly 5 times the size of those deficient in nitrogen. During this 24-day period, the green weight of nitrogen-fed plants increased about 18-fold, whereas that of nitrogen-deficient plants increased less than 4 times. A portion of this increase in nitrogen-deficient plants was probably due to the reserve of unassimilated nitrogen present in these plants at the time they were transferred to the minus-nitrogen treatment.

Table II also shows the virus activity of juice from the nitrogen-deficient plants relative to that of juice from nitrogen-fed plants of the same age at

TABLE II

DATA ON GROWTH OF DISEASED TOBACCO PLANTS AND ON RELATIVE VIRUS ACTIVITY OF THEIR JUICES, WHEN HARVESTED AT INTERVALS AFTER TRANSFER TO THE MINUS-NITROGEN TREATMENT

TIME OF HARVEST: DAYS AFTER TRANSFER TO MINUS-N TREATMENT*	NUMBER OF PLANTS HARVESTED		AVERAGE PER PLANT				VIRUS ACTIVITY† OF N-DEFICIENT PLANTS PER	
			GREEN WEIGHT		VOLUME OF JUICE EXPRESSED			
	+N TREATMENT	−N TREATMENT	N-FED PLANTS	N-DEF. PLANTS	N-FED PLANTS	N-DEF. PLANTS	ML. OF JUICE	PLANT
days			*gm.*	*gm.*	*ml.*	*ml.*	%	%
0	20	....	2.9	.....	2.0	....	.....	....
4	12	12	5.4	4.7	3.0	2.8	100	93
8	10	15	9.5	5.2	5.5	3.7	64	43
12	6	10	16.2	7.5	9.0	4.7	54	28
16	5	10	24.8	7.6	17.8	4.6	87	22
20	5	10	38.2	10.3	26.8	6.4	64	15
24	5	7	52.8	11.1	34.0	7.0	32	7

* Minus-nitrogen treatment started 10 days after inoculation.
† Virus activity relative to that of N-fed plants harvested at same time.

each of the 6 harvests. In each case the activity of the nitrogen-fed plants was assumed equal to 100 per cent. The virus activity per ml. of juice from plants on the minus-nitrogen treatment for 4 days was apparently equal to that of nitrogen-fed plants. After 24 days on the minus-nitrogen treatment, however, the juice from these plants was only $\frac{1}{3}$ as active on a unit volume basis as was that from plants receiving an adequate nitrogen supply. The last column in table II shows the calculated relative virus activity of the nitrogen-deficient plants at each of the harvests. These calculations are based on the volume of juice expressed per plant and the relative virus activity per ml. As shown by the data, the difference in virus activity between the two nitrogen treatments became greater and greater, until by the 24th day the nitrogen-fed plants had nearly 15 times as much virus as did nitrogen-deficient plants of the same age. Possible explanations for this large difference will be discussed in a later section.

Data pertaining to the total-protein content and the virus-protein content of aliquots of juice from the two sets of plants at each harvest are recorded in Table III. In plants receiving nitrogen, the mg. of total protein per ml. of juice increased at first and then decreased slightly. The low protein contents recorded on the 16th and 24th day were probably due in part to the fact that each of these harvest days was preceded by one or more dark, cloudy days. The decrease in carbohydrate synthesis is accompanied by an accelerated hydrolysis of stored proteins. In nitrogen-deficient plants

TABLE III

CONTENT OF TOTAL PROTEIN AND CONTENT AND RELATIVE ACTIVITY OF TWICE-ULTRACENTRIFUGED VIRUS PROTEIN IN JUICE FROM DISEASED PLANTS HARVESTED AT INTERVALS AFTER TRANSFER TO THE MINUS-NITROGEN TREATMENT

Time of harvest: days after transfer to minus-N treatment*	Total protein (mg. per ml.)		Virus protein (mg. per ml.)		Activity† of virus protein from N-def. plants: per unit weight of protein	Activity† of N-def. plants as calculated from virus-protein data
	N-fed plants	N-def. plants	N-fed plants	N-def. plants		
days					%	%
0	11.1	.....	2.7	.....	.....	.....
4	9.5	9.3	3.0	3.5	100	109
8	11.7	8.0	4.4	4.0	71	43
12	12.1	6.1	5.2	3.4	69	24
16	9.2	5.7	3.7	3.1	84	18
20	11.1	5.1	4.5	2.8	88	13
24	9.7	3.7	4.4	2.4	58	7

* Minus-nitrogen treatment started 10 days after inoculation.
† Virus activity relative to that of N-fed plants harvested at same time.

a steady decrease took place in the mg. of total protein per ml. This decrease was apparently brought about by a dilution of synthesized protein with subsequent growth and not to an actual loss of protein. Evidence substantiating this conclusion will be presented later. The virus data show that in nitrogen-fed plants the mg. of virus protein per ml. of juice increased up to the 12th day and then decreased slightly. This slight decrease may be due to an inadequate supply of nitrogen to insure a continuation of the same growth rate in such large plants. The mg. of virus protein per ml. of juice from nitrogen-deficient plants increased slightly at first and then decreased. As in the case of the total protein in these plants, the apparent decrease is only a dilution effect. The composite protein data show that at the last harvest 40 per cent. of the total protein in juice of the nitrogen-fed plants was virus protein, whereas in juice from plants starved for nitrogen, from 60 to 65 per cent. of the total protein was virus protein. This yield of virus is lower than that obtained by STANLEY (**23**), who reported a yield of crystalline virus equal to 80 per cent. of the total protein in the juice. No explanation for this discrepancy has been found.

The virus data discussed so far in table III indicate the virus-protein content in the juices from the two sets of plants but give no information regarding the activity of this virus protein. The fact that virus protein is not broken down does not necessarily imply that the virus is still active, for virus inactivation may not be synonymous with virus disintegration. As LAUFFER and PRICE have recently suggested (**10**), virus inactivation by heat is not identical with thermal denaturation of the virus but may be one of a series of reactions that eventually lead to denaturation. In their heat studies, carried out *in vitro,* virus inactivation proceeded at a faster rate than thermal denaturation.

The 6th column of table III records the biological activity on a unit weight basis of virus protein from the nitrogen-deficient plants relative to that of virus protein from plants receiving the complete nutrient solution. Four days after transfer, the activities per unit weight of virus from the two sets of plants were practically identical. Four days later, virus from the nitrogen-deficient plants was only 71 per cent. as active as that from nitrogen-fed plants. By the 24th day virus from nitrogen-deficient plants was only 58 per cent. as active on a unit weight basis. It might appear as though this value were unusually low in view of the values on the 16th and 20th day, but a later experiment indicated that these two values were not representative and that the 58 per cent. was approximately correct. As will be brought out later, this loss of nearly half the activity was not accompanied by any detectable denaturation or hydrolysis of virus even when nitrogen was withheld from the plant for 24 days.

In the last column of table III is shown the relative virus activity of the entire plant at each harvest, as calculated from the content of virus protein in the juice per plant and the relative activity per unit weight of this virus protein. These calculations show that after the first few days following the transfer from the complete nutrient to the minus-nitrogen treatment, the difference between the virus activities of the two treatments increased very rapidly. By the time the plants had been on the minus-nitrogen treatment for 24 days, juice from these plants was only 7 per cent. as active as that from the nitrogen-fed plants. It is worth noting that these values are essentially the same as those presented in the last column of table II. Although the same entity was measured in both cases, it is interesting that the two methods, one based on measurements with crude juice and the other based on measurements with purified virus, check each other so closely. In this connection it should be pointed out that only a small fraction of the difference between the virus activities of the two groups of plants was due to loss of activity of the virus on the part of the nitrogen-deficient plants. Most of the difference was due to an increase of virus in the nitrogen-fed plants.

In the experiments so far reported, a number of diseased plants was

transferred to the minus-nitrogen treatment 10 days after inoculation. In a second series of experiments, carried out in conjunction with those discussed above, tests were made with plants transferred to the minus-nitrogen treatment 18 days after inoculation. These plants were more than 3 times the size of those changed at the earlier date and were growing much more rapidly at the time of transfer. Representative nitrogen-fed and nitrogen-deficient plants were harvested every 4 days and assayed as in the first series of experiments. The analytical data obtained are recorded in table IV.

It is apparent from these data that the results obtained with the larger plants are very similar to those recorded with the smaller plants. The nitrogen-fed plants increased nearly 8 times in size during the 20-day period; the nitrogen-deficient plants little more than doubled in size. Size was measured by the volume of juice expressed, since this was roughly proportional to the green weight of the plants. The mg. of total protein and of virus protein per ml. of juice remained practically constant in the nitrogen-fed plants but decreased markedly in juice from the nitrogen-deficient plants. At the end of the experiment, 40 per cent. of the protein present in juice from nitrogen-fed plants was present as virus protein, whereas in the juice from nitrogen-deficient plants 55 per cent. of the total protein was accounted for as virus protein. The virus protein in nitrogen-deficient plants was only 70 per cent. as active after the 20-day period on the minus-nitrogen treatment, but no decrease in total mass of virus protein could be detected.

The virus-protein data so far considered have been based entirely on the mg. per ml. of expressed juice and, as such, give only a fragmentary picture of what took place within the plants. In order to fill in the picture, it is necessary to consider total yield of virus protein and of soluble protein per plant as extracted in the plant juice. The yields of virus protein and of total soluble protein in juice from plants on the two treatments at each of the several harvests are represented graphically in figure 1.

As shown by the upper line, the protein content of nitrogen-fed plants increased slowly at first while the plants were small, but as the plants became larger, the protein content increased more markedly. This indicated a rapid synthesis of proteins in these plants. A similar trend was shown by the virus-protein content in these plants as measured at 4-day intervals. The increase appears to be more gradual, whereas in reality the percentage increase was actually much greater. In the 24-day period during the test, the total protein increased about 15 times—from 22 mg. to 330 mg., whereas the virus protein increased 30 times—from 5 mg. to 150 mg.

The graphic representation of data pertaining to the protein content of plants transferred to the minus-nitrogen treatment 10 days after inoculation displayed an entirely different trend. After a small initial rise during

TABLE IV

VIRUS ACTIVITY AND PROTEIN CONTENTS OF JUICE FROM NITROGEN-FED PLANTS AND FROM NITROGEN-DEFICIENT PLANTS AT INTERVALS AFTER INITIATION OF MINUS-NITROGEN TREATMENT, WHICH WAS STARTED 18 DAYS AFTER INOCULATION

TIME OF HARVEST: DAYS AFTER TRANSFER TO MINUS-N TREATMENT	NUMBER OF PLANTS HARVESTED		VOLUME OF JUICE EXPRESSED PER PLANT		TOTAL PROTEIN PER ML. OF JUICE		VIRUS PROTEIN PER ML. OF JUICE		ACTIVITY* OF N-DEFICIENT PLANTS PER		ACTIVITY* OF N-DEF. PLANTS AS ASSAYED WITH	
	N-FED PLANTS	N-DEF. PLANTS	N-FED PLANTS	N-DEF. PLANTS	N-FED PLANTS	N-DEF. PLANTS	N-FED PLANTS	N-DEF. PLANTS	ML. OF JUICE	UNIT WT. OF PROTEIN	CRUDE JUICE	VIRUS PROTEIN
days			*ml.*	*ml.*	*mg.*	*mg.*	*mg.*	*mg.*	%	%	%	%
0	10	...	5.5	.....	11.7	.....	4.4	.....	.....	.....	.....	.....
4	6	6	9.0	9.5	12.1	9.6	5.2	4.4	76	74	80	66
8	5	5	17.8	13.4	9.2	6.9	3.7	3.5	67	79	50	56
12	5	5	26.8	14.4	11.1	6.1	4.5	3.2	67	86	36	33
16	5	7	34.0	15.4	9.7	6.2	4.4	3.6	54	83	24	31
20	5	6	42.2	14.3	10.4	5.6	4.2	3.1	55	68	19	17

* Virus activity relative to that of nitrogen-fed plants harvested at the same time.

the first 8 days on the minus-nitrogen treatment, both the content of soluble protein and that of virus protein remained practically constant during the remainder of the experimental period of 16 days. These data show that the decrease in mg. of protein per ml. of juice, as recorded in table III, was due to a dilution of the protein already synthesized, with subsequent growth, rather than to a loss of this protein, since the protein content of the plant apparently remained constant. Graphs with a similar trend were found when the contents of total protein and virus protein in juice from plants

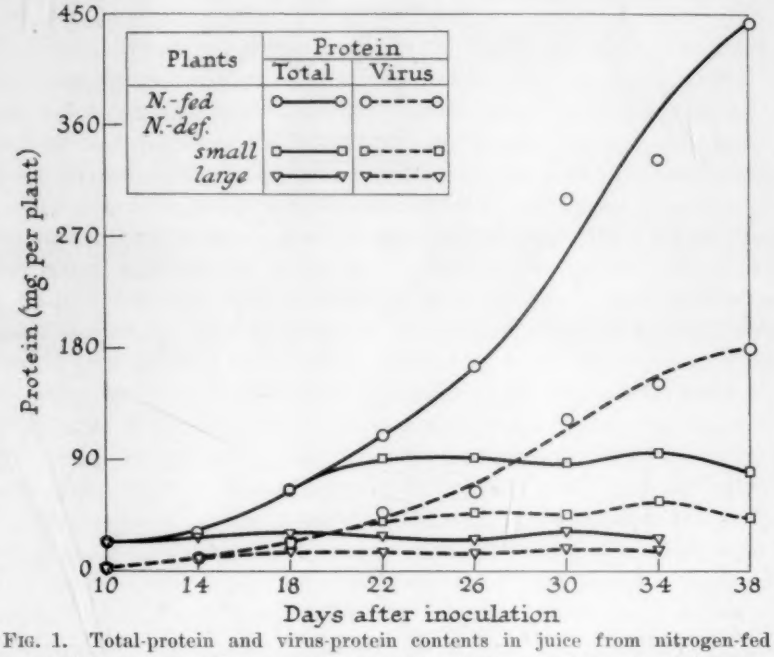

FIG. 1. Total-protein and virus-protein contents in juice from nitrogen-fed and nitrogen-deficient plants at intervals following inoculation with tobacco-mosaic virus.

held on the plus-nitrogen treatment for 18 days before being changed to the minus-nitrogen treatment were plotted.

From the 4 graphs in figure 1, obtained by plotting data from nitrogen-deficient plants, it is possible to draw several tentative conclusions regarding the nitrogen metabolism of a virus-infected plant. In the first place, even a plant suffering from severe nitrogen deficiency is apparently unable to utilize, in the synthesis of its normal proteins, any of the nitrogen in the virus, since its non-virus protein content does not increase and its virus-protein content does not decrease. Some change, however, does take place in the virus in such a plant to render it partially inactive without bringing about any detectable decrease in yield of virus protein per plant. On the

other hand, the virus is apparently unable to utilize any measurable part of the soluble proteins of the plant, for these constituents do not decrease. It is apparent, therefore, that the plant and virus may have competed against each other for the nitrogen absorbed by the plant. Such an hypothesis would explain why a mosaic-diseased plant shows symptoms of nitrogen deficiency at an earlier date than does a healthy plant grown under the same condition. Part of what little nitrogen is available in such plants would be utilized by the virus, rendering the deficiency condition even more severe.

Discussion

Several workers (**1, 13, 17, 23**) have suggested either directly or indirectly that in a mosaic-diseased plant normal proteins may be destroyed or utilized in the formation of virus. This hypothesis was not substantiated by observations in the present study in which it was found that the content of normal proteins in a diseased plant receiving no additional nitrogen did not decrease but remained more or less constant throughout the experimental test period. Although these observations were based on the soluble protein content of the expressed juice, the evidence showed that in the absence of an external supply of nitrogen no further virus multiplication could be detected. This would indicate that no conversion of soluble or insoluble normal proteins into virus took place under these conditions. Virus may multiply, however, at the expense of normal protein by utilizing in its metabolism some of the nitrogen absorbed by the plant before the plant can assimilate such nitrogen in the synthesis of normal proteins.

It has been reported (**13, 17**) that virus protein increases and then decreases with increasing maturity of the diseased plant. The evidence, which has been interpreted to show a destruction of virus, was based on the virus content per gm. of tissue. Similar results have been obtained (**6, 18, 19**) using crude juice. Data obtained in the present paper indicated the same trend, but when these data were calculated on a plant basis it was obvious that, in the absence of further multiplication, the virus content showed no decrease but remained constant. The above-mentioned papers do not contain sufficient growth data to permit such a calculation. The reduction per unit weight or per unit volume is apparently due to a dilution of the virus with subsequent growth of the plant and not to an actual loss or destruction of virus. The data in this paper show that the activity of the virus may decrease under certain conditions. Such an inactivation has not been demonstrated previously.

It is difficult to reconcile the results of the writer with those of RISCHKOV and SMIRNOVA (**17**), who reported that, in tomato plants deficient in nitrogen, virus not only continued to accumulate but even reached a concentration equal to that present in plants supplied normal amounts of nitrogen.

The data presented here (table III and fig. 1) show a much higher virus content in nitrogen-fed plants than in nitrogen-deficient plants.

The partial inactivation of tobacco-mosaic virus in a nitrogen-deficient plant is interesting because of the relative stability of this virus *in vitro*. Two diseases of sugar cane, sereh disease of Java (**7**), and chlorotic streak (**12**), have been cured by heat treatment. The former is believed to be caused by a virus, but some doubt exists as to whether or not chlorotic streak is a virus disease (**2**). Recent experiments with such well recognized viruses as those of aster yellows (**9**), peach yellows, little peach, red suture, and peach rosette (**8**) have definitely established the fact that these viruses can be inactivated *in vivo* by heat. Ross (**18**) has recently reported a marked decrease with age of plant in virus activity of crude juice from tobacco plants diseased with alfalfa-mosaic virus (*Marmor medicaginis* H.). Since this decrease was too rapid to be due to a diluting effect of growth, he concluded that the virus was partially inactivated. No data were reported concerning the content and biological activity of sedimentable virus protein in these plants.

At present writing it is impossible to advance any definite explanation for the inactivation of virus *in vivo*. Ross (**18**) believes that the virus of alfalfa mosaic may have been inactivated by heat, since this virus has a low thermal inactivation point *in vitro* and since the virus never reaches a high concentration in tobacco plants grown during the summer months. Such an explanation would not hold in the case of tobacco-mosaic virus, as this virus is one of the most stable plant viruses so far studied, with a thermal inactivation point of about 90° C. If the virus is a living entity, it is possible that a portion may become avirulent or die out but not be disintegrated, thereby accounting for the loss in activity without a decrease in mass. If the virus is a protein molecule, its biological activity may be dependent on the presence of one or more active groups, and the loss or alteration of such a grouping may occur without a breaking apart of the macromolecule. It is apparent, however, that as far as the plant in a nitrogen-deficient condition is concerned, the virus protein acts as a foreign protein since it is not subject to normal proteolytic hydrolysis by the plant systems. Further work is necessary to elucidate the manner in which virus inactivation takes place in a nitrogen-deficient plant.

Summary

Experiments were carried out to determine in what way tobacco-mosaic virus is affected in a plant deficient in nitrogen. Turkish tobacco seedlings, grown in sand cultures and supplied a complete nutrient solution (nitrogen level = 200 p.p.m.), were inoculated with tobacco-mosaic virus. Ten days after inoculation the plants were divided into two groups; one group (nitro-

gen-fed plants) continued to receive the complete solution and the second group (nitrogen-deficient plants) received a nutrient solution complete in everything but nitrogen. Representative plants from each group were harvested at 4-day intervals and the expressed juice assayed for relative virus activity, total protein, and virus protein.

In nitrogen-deficient plants the virus-protein content as well as the content of soluble plant protein remained practically constant, whereas in nitrogen-fed plants each increased more than 5 times during a 16-day period. Although no decrease in the yield of virus protein in the nitrogen-deficient plants could be detected, its biological activity, however, decreased more than 40 per cent. It is not known how this inactivation was brought about.

As far as the nitrogen-deficient plant is concerned, the virus protein acts as a foreign protein, for the virus was apparently not affected by the normal proteolytic processes of the plant. Even a plant suffering from a severe nitrogen deficiency was unable to use, in the synthesis of its normal proteins, any nitrogen previously utilized by the virus. On the other hand, the virus was unable to utilize any nitrogen tied up in the proteins normally present in a nitrogen-deficient plant, for in the absence of an external supply of nitrogen, no further virus multiplication could be detected.

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RESPONSE OF SEEDLINGS TO VARIOUS WAVEBANDS OF LOW INTENSITY IRRADIATION[1, 2]

ROBERT B. WITHROW

(WITH THREE FIGURES AND TWO PLATES)

The most extensive investigations concerning the influence of radiant energy within the visible spectrum on the growth of shoots of seedlings and storage organs have been conducted by COUPIN (**1**), MACDOUGAL (**2**), PRIESTLEY (**4**), and TRUMPF (**6**). These workers all agree that relatively low intensities of visible radiant energy in the range of 10 foot candles or less induce almost as complete morphological development of the stems and leaves of such shoots as high intensities of many thousands of foot candles. When low intensities are used, the leaves become green, expand into flat lamina, and the portions of the stem first laid down are greatly shortened as contrasted with plants grown in the complete absence of radiant energy where the leaves fail to develop chlorophyll, frequently do not expand, and the stem portions first laid down are relatively long. Many shoots, such as those of the red kidney bean, emerge from the germination medium with a well defined plumular hook, which does not completely disappear in the total absence of visible radiant energy. After the irradiation of the plants with low intensities of visible radiant energy, the plumular hook disappears.

TRUMPF came to the conclusion that chlorophyll synthesis was not directly connected with the mechanism involved in the disappearance of those characteristics associated with growth in the total absence of visible radiant energy. With short low intensity exposures he was able to secure leaf expansion, internodal shortening, and disappearance of the plumular hook without any apparent synthesis of chlorophyll. His results also indicated that it was the longer wavelengths of the visible spectrum that were primarily influential in causing leaf expansion, but stem elongation was not greatly affected by the red. The blue was more effective in inducing shortening of the stem. He used relatively wide bands of the red and blue regions which were balanced to equal readings with a selective radiometer.

This report concerns a preliminary investigation[3] on the influence of spectrally controlled radiant energy on the growth and development of red kidney bean seedlings and other physiologically young plants.

[1] Contributions from the Hull Botanical Laboratory 523.

[2] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

[3] A portion of the investigation was conducted at the Hull Botanical Laboratories, University of Chicago, during the winter of 1937–1938. The remainder of the investigation was conducted in the Department of Horticulture, Purdue University Agricultural Experiment Station, during the year of 1939–1940.

Procedure

The plants were grown in a series of six compartments 32 inches square by four feet high. The compartments were arranged in two units of three compartments per unit in a temperature controlled dark room having facilities for mounting the radiation equipment outside the room so that the lamps had a minimum influence on temperature. Above each compartment was built the radiation system which consisted of a tray of angle iron for the dyed gelatin secondary filters, over which was suspended a 25-inch square glass-bottomed filter cell, eight inches deep, containing distilled water. Where necessary to completely remove red radiant energy, an additional cell of glass, $1\frac{3}{16}$ inch deep by 24 inches square, containing a concentrated solution of copper sulphate, was immersed in the primary filter cell. This system eliminated the necessity of free surfaces of copper sulphate solutions above the plants. The lamp equipment was mounted above the filter cells, and consisted of 1500-watt vapor proof aluminum reflectors of the concentrating type. The details of the lamp equipment and filter systems on five of the six compartments are presented in table I.

TABLE I

DETAILS OF THE RADIATION SYSTEMS

WAVELENGTH LIMITS, Å	RADIATION SOURCE	PRIMARY AQUEOUS FILTER	SECONDARY FILTER DYED GELATIN FILM
BLUE 4047 and 4358 Hg lines	400-watt type H_1 high pressure Hg arc	10 cm. water and 3 cm. – 25 per cent. $CuSO_4 \cdot 5H_2O$	Victoria Pure Blue BO and Crystal Violet
YELLOW-GREEN 5461, 5770 and 5791 Hg lines	400-watt type H_1 high pressure Hg arc	10 cm. water and 3 cm. – 25 per cent. $CuSO_4 \cdot 5H_2O$	Pontamine Fast Yellow 5GL and Orange G
RED 6400 to approximately 9,000 continuous	300-watt gas-filled tungsten filament	10 cm. water	Chrysoidine Y and Crystal Violet— 2 panes $\frac{1}{8}$ inch Corning Aklo plate infrared absorbing glass
FAR RED 6800 to approximately 12,000 continuous	150-watt gas-filled tungsten filament	10 cm. water	Chrysoidine Y and Victoria Pure Blue BO
NEAR INFRARED 7200 to approximately 12,000 continuous	500-watt gas-filled tungsten filament	10 cm. water	Orange G, Pontamine Fast Red 8BL, and Acid Film Green 1854

The sixth compartment served as a dark plot. In some of the experiments, the near infrared treatment was eliminated and a second dark plot included. Figure 1 is a graph of the transmission spectra of the filter systems used.

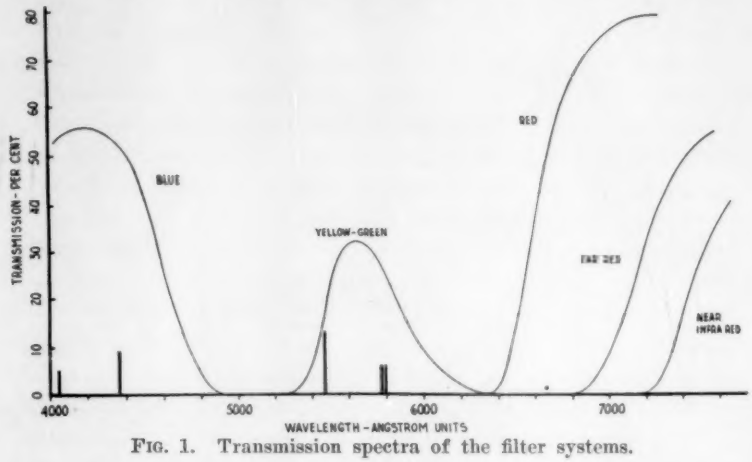


FIG. 1. Transmission spectra of the filter systems.

The energy was balanced with a thermopile which was mounted 20 cm. above the gravel surface and covered with a filter cell containing 18 mm. of ferrous ammonium sulphate saturated at 22° C., as described by PFUND (3). This filter efficiently absorbs radiant energy beyond about 8000 Å. with relatively little absorption within the range of the visible spectrum. The radiant energy for each plot was adjusted to the values presented in the tables of results. The final adjustments were obtained by placing narrow strips of black paper across the filter panes. It was assumed tentatively that the energy beyond 8000 Å. has little effect, this assumption being based upon a preliminary experiment in which no effects were obtained with radiant energy beyond this limit. For this preliminary experiment an irradiance of 1500 ergs per sq. cm. per second from an incandescent lamp source was filtered with 10 cm. of distilled water and a 3.5-mm. Corning no. 254 infrared transmitting glass with transmission from 8000 Å. to 12000 Å. The results secured indicated that no appreciable effects on red kidney bean occur when the plants were irradiated with these wavelengths.

The plant material was grown in subirrigation gravel culture beds, 6 inches deep by 28 inches square. Tap water was supplied to the beds automatically every four hours by means of a time switch and a centrifugal pump. A pair of small fans circulated air up over the beds at a sufficiently rapid rate to maintain the temperature of the compartments the same as that of the room. The irradiation was applied continuously with no dark periods, except for the experiments reported in table VI, where the irradia-

tion was applied both continuously and for ten per cent. of the daily cycle, or 2.4 hours daily. Where high humidities were used, the air was kept supplied with water vapor from a small spray unit placed in front of a large fan. The temperature was maintained at 25° C.

Seeds of red kidney bean (*Phaseolus vulgaris*) were selected for uniform size. The variation in total seed weight per plot in any one experiment was not more than 0.5 per cent. There was considerable variation, however, in size of seed selected for the different experiments. The seeds were sown at a depth of one inch with a spacing of 2×2 inches. As soon as the plumular hook appeared above the gravel, the irradiation was applied. The plants were harvested 14 days from seeding.

Length measurements were made of the hypocotyl and first and second internodes. The internodes in this study were numbered from the cotyledon up the stem in the order of development. Fresh and dry weight were taken for the hypocotyl, first internode, cotyledons, first leaves and roots. Those portions of the plant above the first leaves were weighed as one fraction. The plant material was dried for 18 hours at 100° C. in a forced draft oven.

The leaf pigments were extracted with 100 ml. of acetone from 5 gm. of fresh leaf tissue. The relative chlorophyll concentration was then determined with a visual spectrophotometer from the specific transmissive index of the acetone solution using the 6630 Å. absorption band of chlorophyll.

Where transfers were made to the greenhouse from the radiation rooms, the greenhouse temperatures were maintained at approximately 19° C. during the day and 13° C. during the night. The short day condition was 9 hours long. Two long day conditions were used, one with 9 hours daylight supplemented with 15 hours of irradiation with 10 foot candles from an incandescent lamp or 9000 foot candle minutes nightly. The second long day treatment consisted of 9 hours daylight supplemented with equal energy of 9000 foot candle minutes supplied by intermittent irradiation with 200 foot candles on for 5 per cent. of the time or 1.5 minutes half-hourly. These greenhouse irradiation conditions were used because they were already in operation in connection with other experiments.

Seeds of pea (*Pisum sativum*), variety Little Marvel, were selected for uniform weight and sown with the same spacing and at the same depth as the red kidney bean, using the same wavebands and temperature. Length measurements were made of six internodes above the cotyledons and fresh and dry weights of leaves, stems, roots, and cotyledons were obtained. The plants were dried at 100° C. for 18 hours.

Pea, maize (*Zea mays*) and soybean (*Soya max*) seeds and small potato tubers (*Solanum tuberosum*) were selected for uniform size and these, together with tomato (*Lycopersicum esculentum*) and cocklebur (*Xanthium*

pennsylvanicum) seed, were grown under far red treatment (6800 Å. to 12000 Å.), and in complete darkness, all other experimental conditions being similar to those outlined above.

Results and discussion

Of the five bands of radiant energy used, the yellow-green and red caused the most pronounced morphological effect as contrasted with plants grown in the total absence of radiant energy. The far red band produced almost as great an effect on the development of the plants as the yellow-green and red, but the blue and near infrared bands had much less effect. On the basis of the data in the tables, the near infrared appears to be almost as effective as the blue, but it should be noted that the energy of the near infrared was 15 times that of the other plots.

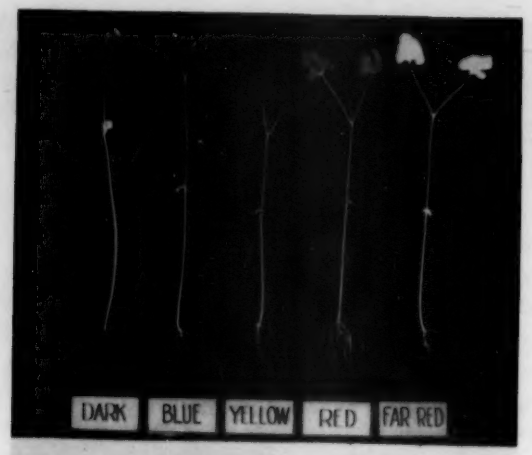
With the yellow-green and red bands of radiant energy, the hypocotyl and first internode lengths of red kidney bean were shortened (tables II, III, IV, and VI) and there was an increased development of the epicotyl above the first internode (plate I and fig. 2). Marked leaf expansion occurred under these wavelengths together with the rapid disappearance of the plumular hook.

Under the blue, there was some shortening of the hypocotyl and first internode. The apical hook originating as a plumular hook, moved toward the tip of the plant and at the time of harvest had become a petiolar hook of the first leaves. Little leaf expansion occurred. Under the near infrared, the plumular hook disappeared entirely and some leaf expansion took place.

The dry weights show that all of the spectral regions, especially the red, accelerated the movement of the food reserves from the cotyledons into the extremities of the plant, the accelerated movement taking place both up the stem into the terminal portions of the shoot, and down into the root system. The dry weights of these parts increased considerably over similar fractions of plants grown in complete darkness or in the other spectral regions (fig. 3). In plants grown in the complete absence of radiant energy, the hypocotyl and first internode, both adjacent to the cotyledonary node, received the major portion of the food reserves translocated from the cotyledons.

The percentage of dry matter was at a maximum in the longer wavelength treatments. The top-root ratio was at a minimum under the same conditions as a result of the accelerated tendency of the food reserves to move into the root system.

This whole series of reactions which was brought about most effectively by the longer wavelengths of the visible spectrum does not appear to be directly related to respiration since, as shown in table II, there does not appear to be any significant difference in loss of dry matter by the plants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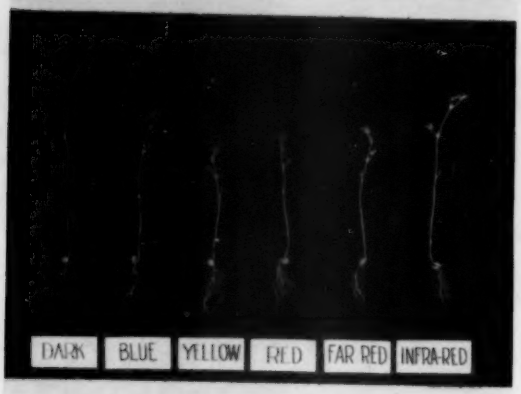
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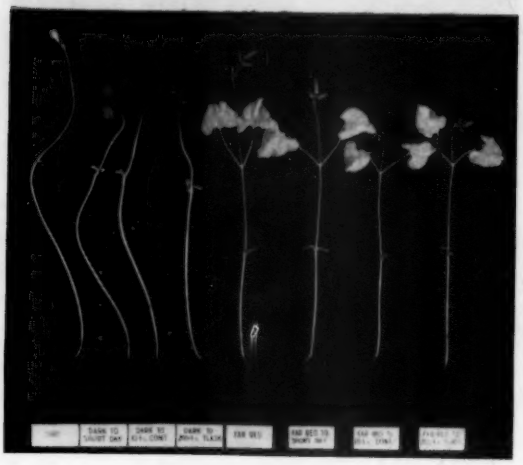


as calculated from the difference between the total dry weight at harvest and the dry seed weight minus the seed coat.

Neither do these responses appear to be related to the amount of chlorophyll present in the leaf. Table II presents the relative concentration of chlorophyll per unit of fresh weight of leaf tissue. The highest chlorophyll

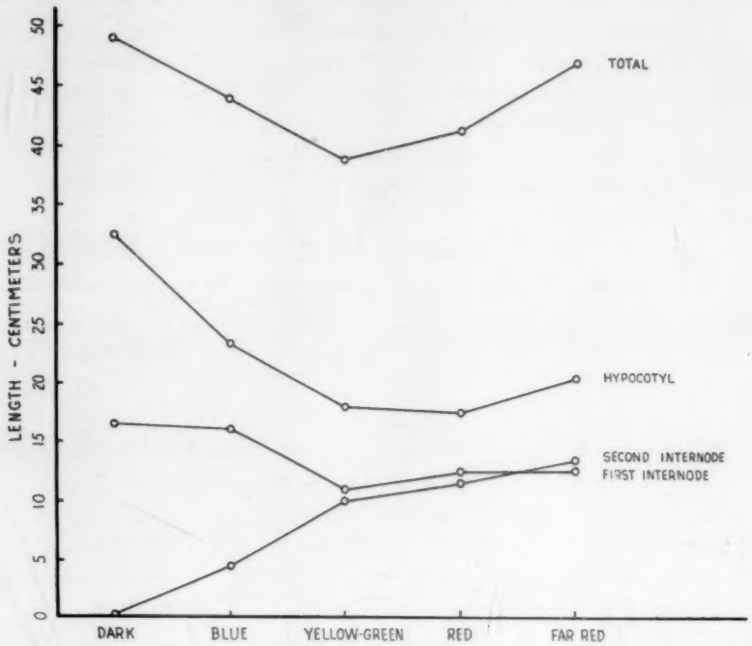


FIG. 2. Stem lengths of red kidney bean seedlings exposed to 500 ergs per sq. cm. per second of filtered radiant energy of the regions indicated.

PLATE III

EFFECT OF WAVELENGTH OF RADIATION ON THE GROWTH RESPONSE OF BEAN AND PEA

TOP.—Bean plants showed the maximum leaf expansion, development of the second internode, and the shortest hypocotyls in the yellow, red and far red. In the far red, only a trace of chlorophyll developed, whereas in the red, yellow and blue, the leaves were quite green. The development of the plants under the blue was similar to that of the plants grown in the dark with the exception of the presence of chlorophyll in those portions of the leaves exposed to the blue irradiation.

CENTER.—Pea, showing relationships similar to those exhibited by bean, although the differences occurring under the various spectral regions are not as marked.

BOTTOM.—Bean plants transferred from the dark and far red treatments to the greenhouse. The dark conditioned plants expanded their leaves and developed chlorophyll with a denser green color apparent in the long day treatment than in the short day condition. The plants conditioned by the far red failed to develop chlorophyll in the leaves previously exposed to the far red radiation. Those leaves in the bud at the time of transfer unfolded and developed chlorophyll.

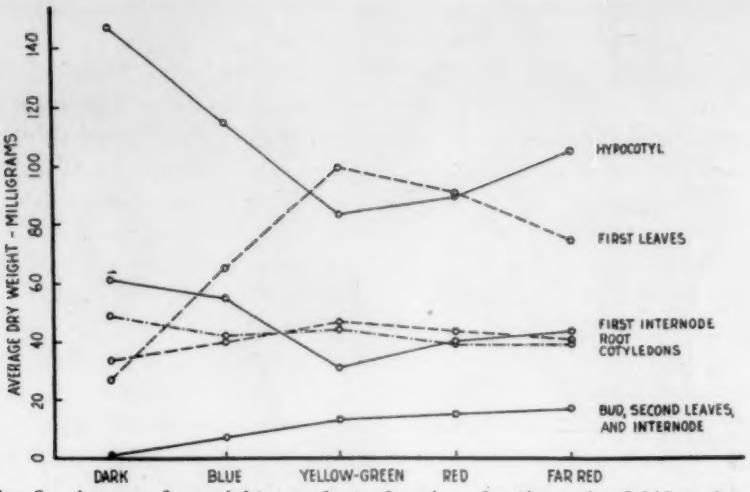


FIG. 3. Average dry weight per plant of various fractions of red kidney bean.

TABLE II

THE GROWTH RESPONSE OF RED KIDNEY BEAN SEEDLINGS* TO A CONTINUOUS IRRADIANCE OF 500 ERGS PER SQ. CM. PER SECOND AT 25° C.

PLANT FRACTION	DARK	BLUE	YELLOW-GREEN	RED	FAR RED
	LENGTH				
	cm.	*cm.*	*cm.*	*cm.*	*cm.*
Second internode	0.1	4.5	10.0	11.4	13.6
First internode	16.6	15.9	11.0	12.5	12.8
Hypocotyl	32.5	23.6	17.9	17.5	20.3
Total	49.2	44.0	38.9	41.4	46.7
	DRY WEIGHT				
	mg.	*mg.*	*mg.*	*mg.*	*mg.*
Bud, second leaves and internode	1	7	13	15	17
First leaves	27	65	99	90	74
First internode	61	55	31	40	43
Hypocotyl	147	114	83	89	104
Cotyledons	49	42	44	39	39
Root	34	40	46	43	40
Total	319	323	316	316	317
Percentage dry matter	4.9	5.6	6.0	6.0	5.9
Mg. respired	129	125	132	131	130
Top-root ratio	6.9	6.0	4.9	5.5	6.0
Relative chlorophyll concentration	0	14.5	27.0	16.5	0.9

* Plants per treatment: 100.

concentration appeared in the yellow-green. Since the leaves were not expanded in the blue, only a relatively small amount of surface was exposed to the radiant energy. In these exposed regions the green coloration appeared to be more intense than in any of the other plots, even though the extraction data, based on total leaf weight, does not show this.

The plants irradiated with the far red were consistently nearly as fully developed morphologically as those in the yellow-green and red plots, but the leaves contained only a trace of chlorophyll. SAYRE (5) has reported that chlorophyll is not formed to any appreciable extent by wavelengths longer than 6800 Å. The results of this experiment are in harmony with his conclusion. Chlorophyll synthesis therefore would not appear to be directly connected with the morphological responses obtained, since almost the maximum response occurred in the far red where almost no chlorophyll was present and a minimum response in the blue where a relatively high chlorophyll concentration obtained. These conclusions are in accord with those of TRUMPF who obtained similar morphological development without the synthesis of chlorophyll when the seedlings were treated with very short exposures to white irradiation.

TABLE III

THE GROWTH RESPONSE OF RED KIDNEY BEAN SEEDLINGS* TO A CONTINUOUS IRRADIANCE OF 100 ERGS PER SQ. CM. PER SECOND AT 25° C. AND 25 PER CENT. RELATIVE HUMIDITY

PLANT FRACTION	DARK	BLUE	YELLOW-GREEN	RED	FAR RED	INFRA-RED†
	LENGTH					
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
Second internode	0.4	4.1	9.3	9.5	8.9	5.8
First internode	21.5	15.3	15.2	14.4	15.6	18.3
Hypocotyl	23.6	20.6	15.4	15.0	16.1	21.6
Total	45.5	40.0	39.9	38.9	40.6	45.7
	DRY WEIGHT					
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Bud, second leaves and internode	1	6	11	13	11	9
First leaves	20	36	43	45	44	41
First internode	63	53	45	43	49	58
Hypocotyl	91	92	73	73	81	90
Cotyledons	70	54	62	56	60	54
Root	28	28	33	32	37	29
Total	273	269	267	262	282	281
Percentage dry matter	5.2	5.9	6.4	6.1	6.5	5.9
Top-root ratio	6.2	6.7	5.2	5.5	5.0	6.8

* Plants per treatment: 40.
† Adjusted to 1500 ergs per sq. cm. per second.

Several minor experiments were conducted on the plants irradiated with wavelengths longer than 6800 Å. as obtained in the far red treatment. Since the expanded leaves of these plants contained very little chlorophyll, and were yellowish in color, they were transferred to the greenhouse to full sunlight nine hours daily in order to determine whether chlorophyll would be synthesized normally in these plants. It was found that fully expanded leaves previously irradiated with the far red did not synthesize any chlorophyll in seven days time (plate I). At the end of a week the leaves were still alive but completely lacking in green coloration. The young leaves which unfolded after the transfer to the greenhouse developed chlorophyll quite normally. Plants taken from the dark to the greenhouse expanded their leaves which became green. In this case, the color was more intense in the long days than in the short days, as indicated in the photograph. Some of the plants from the far red were placed in tap water and some in a complete nutrient solution, but the effect was the same in both cases.

TRUMPF likewise obtained somewhat similar results with his plants which developed expanded leaves containing no chlorophyll under the influence of short exposures to white irradiation. His plants failed to develop chlorophyll in the older leaves on transfer to the greenhouse.

TABLE IV

THE GROWTH RESPONSE OF RED KIDNEY BEAN SEEDLINGS* TO A CONTINUOUS IRRADIANCE OF 100 ERGS PER SQ. CM. PER SECOND AT 25° C. AND 75 PER CENT. RELATIVE HUMIDITY

PLANT FRACTION	DARK	BLUE	YELLOW-GREEN	RED	FAR RED	INFRA-RED†
	LENGTH					
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
Second internode	2.3	5.5	11.4	11.7	9.9	5.5
First internode	23.8	16.5	16.4	14.6	15.5	18.2
Hypocotyl	21.6	18.5	14.6	15.4	16.2	20.7
Total	47.7	40.5	42.4	41.7	41.6	44.4
	DRY WEIGHT					
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Bud, second leaves and internode	2	7	16	15	12	7
First leaves	24	45	56	55	50	42
First internode	64	51	44	37	43	52
Hypocotyl	81	79	74	73	75	83
Cotyledons	46	44	40	42	45	46
Root	31	35	42	45	41	38
Total	248	261	272	267	266	268
Percentage dry matter	4.8	5.3	5.6	5.7	5.8	5.4
Top-root ratio	5.5	5.2	4.5	4.0	4.4	4.8

* Plants per treatment: 25.
† Adjusted to 1500 ergs per sq. cm. per second.

TABLE V

THE GROWTH RESPONSE OF PEA, VARITY LITTLE MARVEL, SEEDLINGS* TO A CONTINUOUS IRRADIANCE OF 100 ERGS PER SQ. CM. PER SECOND AT 25° C. AND 75 PER CENT. RELATIVE HUMIDITY

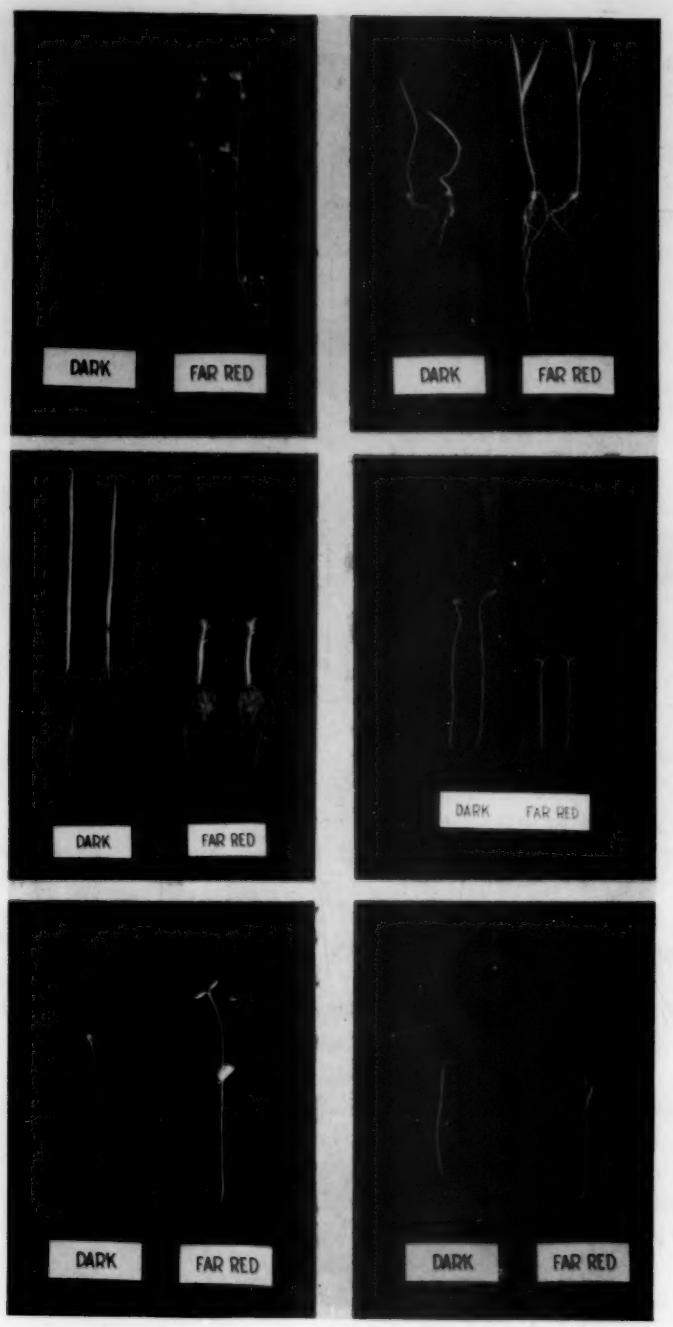
PLANT FRACTION	DARK	BLUE	YELLOW-GREEN	RED	FAR RED	INFRA-RED†
	LENGTH					
	cm.	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
Internode VI	0.4	0.8	0.9	0.6	1.1	1.1
Internode V	6.7	3.5	2.8	2.3	2.9	3.9
Internode IV	12.2	4.6	3.4	2.9	3.8	6.0
Internode III	9.9	6.0	3.2	3.6	4.1	6.1
Internode II	2.5	3.1	2.0	2.5	2.5	3.0
Internode I	3.9	3.8	3.1	3.5	3.7	3.7
Total	35.6	21.8	15.4	15.4	18.1	23.8
	DRY WEIGHT					
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
Leaves	9	22	28	26	27	21
Stems	63	41	32	30	37	41
Cotyledons	46	44	44	37	43	41
Roots	24	24	27	25	27	24
Total	142	131	131	118	134	127
Percentage dry matter	5.1	5.4	6.0	5.7	5.7	5.1
Top-root ratio	3.0	2.6	2.3	2.3	2.4	2.6

* Plants per treatment: 30.
† Adjusted to 1500 ergs per sq. cm. per second.

Data given in table II were secured at 500 ergs per sq. cm. per second and in table III at 100 ergs per sq. cm. per second. These show the same general trend in all cases. It was not practical to reduce the intensity much further than 100 ergs and therefore intermittent irradiation was used with the lamps on for 2.4 hours daily so as to reduce the total daily radiant energy to 10 per cent. of the continuous irradiation. Height results for bean and pea under these conditions are given in table VI. It can be seen here that the differences were considerably less marked with intermittent irradiation, but there was still a great deal of influence on the development of the plants.

Tables III and IV give comparative data for results secured at 25 per cent. and 75 per cent. relative humidity. The same trend of morphological development was observed in both cases although a slightly higher percentage of dry matter occurred at the lower humidity, and the leaf expansion was not as great. The actual weight and height figures cannot be directly compared, however, since the initial seed weights were not the same.

Several experiments were run with the Little Marvel variety of pea and a set of typical results are given in table V. The weight results were similar to those obtained with bean. The leaves were somewhat more fully ex-


DARK
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panded under the longer wavelengths and the total height was less. Internode lengths show that as the internodes become further removed from the cotyledons, they become shorter under all wavelengths of radiant energy, with the greatest shortening with the yellow-green, red, and far red (plate III). The leaves were heavier under the longer wavelengths, and the dry weights of the stems less.

Since bean plants develop with expanded leaves under radiant energy of wavelengths longer than 6800 Å. without appreciable development of chlorophyll, a number of other species of plants was grown without radiant energy and with this region of the spectrum applied continuously at 2000 ergs per sq. cm. per second. As can be seen from the photographs presented in plate II, the responses of all dicotyledonous plants were similar to those of the bean, with increased leaf expansion, shortened hypocotyl, and the disappearance of the plumular hook where either was present. In the case of maize, leaf expansion was greatly increased under the far red. Only a trace of green coloration could be observed in any of these plants.

Red kidney bean plants were grown in a preliminary experiment using a complete nutrient solution containing both nitrate and ammonium nitrogen. The same type of results was secured with a complete nutrient solution as with tap water.

Summary and conclusions

Seedlings of red kidney bean (*Phaseolus vulgaris*) and pea (*Pisum sativum*) were grown in gravel culture, subirrigated with tap water, in the complete absence of visible and near visible radiant energy, and irradiated with 500 and 100 ergs per sq. cm. per second of five bands of visible and near infrared radiation including: blue (4047, and 4358 Å. Hg lines); yellow-green (5461, 5770, and 5791 Å. Hg lines); red (6400 to approximately 9000 Å. incandescent); far red (6800 Å. to approximately 12000 Å incandescent); and 1500 ergs per sq. cm. per second of near infrared (7200 Å to approximately 12000 Å incandescent).

Seedlings of pea, maize, soybean, tomato, and cocklebur, and sprouts of

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PLATE IV

FAR RED RADIATION

Influence of low intensity far red radiation (6800–12000 Å.) on the morphological development of pea (top left), maize (top right), potato (center left), tomato (center right), soybean (bottom left), and cockle bur (bottom right). The far red caused the disappearance of the plumular hook in the dicotyledonous plants, a shortening of the hypocotyl where it was present, and increased the leaf expansion and development of the apical bud. Only a trace of chlorophyll occurred in the plants treated with the far red radiation.

TABLE VI

THE HEIGHT RESPONSE OF RED KIDNEY BEAN AND PEA, VARIETY LITTLE MARVEL, SEEDLINGS TO RADIANT ENERGY APPLIED CONTINUOUSLY AND FOR 10 PER CENT. OF THE DAILY CYCLE AT 25° C.

PLANT	IRRADIATION TREATMENT	PLANT FRACTION	DARK	BLUE	YELLOW-GREEN	RED	FAR RED	INFRA-RED*
Red kidney bean	Continuous	Second internode	2.3	5.5	11.4	11.7	9.9	5.5
		First internode	23.8	16.5	16.4	14.6	15.5	18.2
		Hypocotyl	21.6	18.5	14.6	15.4	16.2	20.7
		Total	47.7	40.5	42.4	41.7	41.6	44.4
	10 per cent. daily cycle†	Second internode	1.5	4.4	8.0	10.9	4.9	3.1
		First internode	23.2	21.7	21.2	17.4	21.0	22.5
		Hypocotyl	23.0	20.2	17.3	19.7	19.8	22.5
		Total	47.7	46.3	46.5	48.0	45.7	48.1
Pea	Continuous	Total	35.6	21.8	15.4	15.4	18.1	23.8
	10 per cent. daily cycle†	Total	35.6	27.3	24.0	23.4	28.2	33.2

* Adjusted to 1500 ergs per sq. cm. per second.

† All heights multiplied by factor 1.07 for bean, and 1.10 for pea, in order to adjust the total height of the dark plants to the same value for continuous irradiation and for irradiation applied for 10 per cent. of the daily cycle.

potato were grown in the absence of radiant energy and irradiated with 2000 ergs per sq. cm. per second of far red irradiation.

With red kidney bean, the yellow-green and red bands caused the most pronounced morphological differences as compared with plants grown in the complete absence of radiant energy. The far red produced almost as great an effect, but the blue and high intensity infrared were much less effective. The longer wavelengths of the visible spectrum caused a marked increase in leaf size and expansion and a shortening of the hypocotyl and first internode. The dry weights of the hypocotyl and first internode were greatly reduced, whereas the dry weights of the root, first leaves and the epicotyl above the first leaves were materially greater than those of the dark conditioned plants.

The data presented indicate that in the absence of radiant energy the major portion of the reserve material translocated from the cotyledons remained in those parts of the plant immediately adjacent to the cotyledonary node, *i.e.*, the hypocotyl and the first internode. The longer wavelengths of the visible spectrum increased the total amount of reserves translocated from the cotyledons, and also greatly increased the proportion translocated beyond the hypocotyl and first internode, that is, to the epicotyl above the first internode, and to the roots. The accelerated movement was considerably greater toward the apical portions of the stem than to the roots.

It is concluded that the process is not directly related to chlorophyll synthesis, since a minimum morphological response was produced by the blue region with a strong development of chlorophyll, and almost a maximum morphological response occurred in the far red where only a trace of chlorophyll appeared.

Pea gave much the same type of response as red kidney bean as to total stem length, leaf expansion and weight and translocation of material from the cotyledons.

The far red had essentially the same type of effect on maize, soybean, tomato, cocklebur, and potato as it had on red kidney bean. The far red region caused a disappearance of the plumular hook of the dicotyledonous plants, a shortening of the hypocotyl where present, an increased leaf expansion and an increased size of roots. Only a trace of green color was apparent in the leaves of any of these plants under the far red.

The author wishes to express his sincere appreciation to Dr. C. A. SHULL for his many helpful suggestions during this investigation.

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ABSORPTION AND UTILIZATION OF RADIOACTIVE CARBON DIOXIDE BY SUNFLOWER LEAVES

JAMES H. C. SMITH AND DEAN B. COWIE[1]

(WITH TWO FIGURES)

Introduction

The absorption of carbon dioxide by leaves is one of the important steps in the over-all process of photosynthesis. Leaves, although unilluminated, absorb carbon dioxide reversibly from the surrounding atmosphere. Some leaves also contain considerable quantities of carbon dioxide combined as carbonates and bicarbonates. Both of these properties are especially marked in sunflower leaves.

Analysis of carbon dioxide absorption by sunflower leaves has indicated that absorption takes place in at least three different ways: by physical solution in the water of the sap; by chemical reaction with buffer substances dissolved in the sap; and by insoluble alkaline earth carbonates (**7**). To what extent each of these reactions participates in the total absorption by the living leaf and to what extent the absorption of carbon dioxide in the absence of light concerns the photosynthetic process were not ascertainable. When radioactive carbon became accessible a means was provided for investigating these relations, and it is the purpose of this paper to report some of the results of such an investigation.

Methods and results

PREPARATION AND MEASUREMENT OF RADIOACTIVE CARBON DIOXIDE

The radioactive carbon was made by bombarding boron oxide with 2 MEV deuterons in the high-voltage equipment of the Department of Terrestrial Magnetism of the Carnegie Institution of Washington (**8**). The oxides of carbon were removed from the boron oxide glass by heating and were converted completely into carbon dioxide by combustion over copper oxide (**9**). Ordinary carbon dioxide was used as carrier for the radioactive gas. The radioactive carbon dioxide was purified by solution in potassium hydroxide followed by liberation with lactic acid. Extraneous gases were removed from the alkaline solution by evacuation before addition of the acid. In this way samples of pure carbon dioxide were obtained.

In this paper the term "radioactive carbon dioxide" is used to denote carbon dioxide to which a sample of radioactive carbon dioxide has been added for indicator purposes. The radioactivity of the resulting mixture

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enables the reactions of this particular sample of gas to be followed quantitatively. The term radioactive is preferred to the terms "labeled" or "tagged," since the two latter terms are not specific and apply to samples containing mass isotopic indicators as well as radioactive isotopic indicators.

For determination of the radioactivity of the carbon dioxide, a measured quantity of the gas was drawn into an evacuated flask containing 5.00 ml. of N potassium hydroxide. The alkaline solution containing the radioactive carbonate (4.00 ml.) was pipetted into a hard rubber cup, 4.5 cm. in diameter and 0.5 cm. deep. After being covered with a 0.1-mm. aluminum foil, the cup was placed in position under an ionization chamber attached to a LUTZ-EDELMANN electrometer (1) by means of which the radioactivity was measured.

In order to increase the reproducibility of the determinations of radioactivity, the inside of the cup was paraffined to prevent creeping of the solution and the surface of the alkaline solution was touched with a wire moistened with olive oil to eliminate certain objectionable surface-tension effects.

The radioactivities of the various fractions of carbon dioxide obtained during the course of an experiment were compared by referring them to a common time by means of the half-life constant, 20.35 minutes. This half-life constant was determined by a series of separate experiments under the conditions just described and is probably accurate to within $\pm$ 0.23 minutes.

CARBON DIOXIDE EXCHANGE IN SUNFLOWER LEAVES IN THE DARK

From the previous work (7) it appears that sunflower leaves contain a considerable quantity of carbon dioxide combined in the form of salts. These may constitute a reservoir of carbon dioxide potentially available for photosynthesis. In order to determine whether the carbon dioxide absorbed from the surrounding atmosphere becomes a part of this reservoir the following experiment was performed.

Sunflower leaves (10.00 gm.) were cut into pieces with an area of about one square inch. These pieces were put into a reaction flask attached to a manometer-pump system similar to that previously described (7). The flask was immersed in a constant-temperature water bath maintained at 15.0° $\pm$ 0.1° C. After the flask containing the leaves had been exhausted by means of a SPRENGEL pump, a known volume of radioactive carbon dioxide was admitted to the flask. (The apparatus used in these experiments differed from the one previously described (7) in that the gas introduced into the reaction vessel was measured volumetrically rather than manometrically.) By comparing the change in pressure produced with the change caused by introduction of the same volume of an insoluble gas (nitrogen) the amount of carbon dioxide absorbed by the leaves was calcu-

lated (table II). The amount of carbon dioxide which reacted chemically (table II) was estimated by subtracting the amount physically dissolved in the sap of the leaves from the total amount absorbed. The amount of carbon dioxide physically dissolved by the sap of the sunflower leaves was calculated from the water content of the leaves, the partial pressure of the carbon dioxide in the flask at equilibrium, and the solubility of the carbon dioxide in the sap, *viz.*, 0.926 ml. per gm. of water, per atmosphere of carbon dioxide pressure, volume reduced to standard conditions (7).

The carbon dioxide pumped from the reaction flask by means of the SPRENGEL pump and collected in another gas buret (table I) accounted for all of the carbon dioxide admitted initially to the reaction flask. The radioactive carbon dioxide recovered by pumping was less than the amount introduced. In all cases the amount retained is a larger proportion of the amount introduced than the proportion retained by an equal weight of water. These results indicate that an exchange of carbon dioxide takes place within the leaves.

By acidification of the leaves, it was shown that some of the radioactive carbon dioxide absorbed from the atmosphere had replaced carbon dioxide in carbonates and bicarbonates. After the absorption experiments had been completed, and as much as possible of the carbon dioxide had been removed by evacuation, the leaves were treated with 10 ml. of 6 N hydrochloric acid.

TABLE I

RETENTION OF CARBON DIOXIDE BY SUNFLOWER LEAVES AND BY WATER

MATERIAL TESTED	PRESSURE CO_2	VOLUME INTRODUCED	VOLUME REMOVED	RADIOACTIVITY INTRODUCED	RADIOACTIVITY REMOVED	PERCENTAGE RETAINED
	atm.	*ml.*	*ml.*	*units*†	*units*†	
Sunflower leaves, 10 grams fresh weight	0.2620*	13.51	13.85	2429	2289	5.76
	0.2645*	14.08	14.73	1132	1042	7.95
	0.2870	14.72	15.13	748	728	2.76
	0.5608	27.79	28.28	876	790	9.75
	0.6124*	30.96	31.57	7824	6691	14.48
	0.6520*	32.16	32.72	5427	5267	2.95
	0.7139	35.34	35.55	1155	1074	7.01
Water, 10 ml.	0.2351	11.12	11.17	1511	1484	1.82
	0.4686	22.15	22.34	6322	6620	−4.72

* The measuring cell in these experiments was covered with cellophane.

† The units of radioactivity are entirely arbitrary and have no absolute significance. The units used in any one experiment are consistent and comparable.

The carbon dioxide liberated (table II) was pumped from the reaction flask and its radioactivity determined. This gas contained considerable quantities of radioactive carbon dioxide (table II) which must have been retained by the leaves in the form of carbonates and bicarbonates.

Separate tests showed that 10.00 ml. of water when placed in the reaction vessel retained only a small quantity of the radioactivity introduced into the reaction system. The test was made by pumping from the water as much as possible of the radioactive carbon dioxide, adding sodium carbonate to the water, and liberating the carbon dioxide with hydrochloric acid. This carbon dioxide was recovered by pumping, and in two experiments was shown to contain 1.47 and 0.21 per cent. of the activity initially introduced, corresponding to 0.16 and 0.05 ml., respectively.

Still further evidence for the exchange of carbon dioxide within the leaf was the observation that leaves which had been saturated with radioactive carbon dioxide retained their radioactivity after evacuation and after standing in the dark for as long as two hours.

One sample of leaf discs which had been saturated with radioactive carbon dioxide contained 82 units of radioactivity. After evacuation and flushing five times with ordinary carbon dioxide, this sample still contained 23 units of radioactivity, or 27 per cent. of the amount initially absorbed. This approximates the amount retained in the absorption experiments the results of which are recorded in table II, *i.e.*, 22.7 per cent. (average value).

Another leaf sample, saturated with radioactive carbon dioxide and stored in the dark for 129 minutes, possessed the following activities: initial activity, 1195 units; activity after storage, 212 units; activity after acidification, 9 units. That most of this activity was retained in the leaf as carbonate or bicarbonate is shown by the large loss of activity on treatment with hydrochloric acid.

From the results reported in table II it is apparent that the amount of carbon dioxide retained closely parallels the amount of carbon dioxide that had reacted chemically in the leaf. If a reasonable explanation of the mechanism of the exchange can be obtained it may provide insight into the carbon-dioxide-absorption process, for these two processes are undoubtedly closely allied.

A reasonable conception of the absorption and exchange processes has been obtained from the experiments on the reversible absorption of radioactive carbon dioxide. According to this conception, of the total carbon dioxide absorbed (a), a portion (r), combines chemically with certain constituents within the leaf. Part of this combines with buffer substances dissolved in the sap of the leaf, and another part (fr) reacts with insoluble carbonates. The latter is thus dissolved in the leaf sap. There is already present in the leaf sap a residual amount of bicarbonate (b), with which the absorbed carbon dioxide and bicarbonate ion formed by dissolution of the insoluble carbonates, are pooled. The amount of exchange (e) observed is the ratio of the amount of carbon dioxide retained on evacuation to the total amount in the pool at equilibrium multiplied by the total amount absorbed.

TABLE II

THE EXCHANGE OF CARBON DIOXIDE IN SUNFLOWER LEAVES

PRESSURE CO_2	VOLUME OF CARBON DIOXIDE				EXCHANGE CALCULATED
	ABSORBED	REACTED	RETAINED†	LIBERATED	
atm.	*ml.*	*ml.*	*ml.*	*ml.*	*ml.*
0.262*	3.46	1.50	0.95	.....	0.92
0.265*	3.95	1.81	0.69	19.2	1.07
0.287	3.73	1.59	0.94	28.3	0.97
0.561	6.37	2.23	1.54	28.3	1.38
0.612*	7.51	3.10	1.76	33.2	1.80
0.652*	7.37	2.53	1.48	19.3	1.55
0.714	8.15	2.87	1.72	32.4	1.74

* Measurements of radioactivity made with cup covered with cellophane window. In others an aluminum window was used.

† The amount retained was calculated from the amount of radioactivity liberated with acid after removing as much as possible of the carbon dioxide by pumping.

The algebraic equation representing this conception of the exchange process is

$$\text{(A)} \qquad \frac{b+fr}{b+fr+a}\,a = e$$

The amount of exchange calculated by this expression agrees well with the amount of exchange observed (table II). In the algebraic equation the values assigned to b and f are 0.20 and 0.70 ml., respectively. The values of a and r are taken from table II.

While direct evidence concerning the chemical nature of the insoluble substance in the living leaf which reacts with carbon dioxide (represented by the term fr) is not available, its chemical nature may be deduced indirectly. Previous work on killed sunflower leaves indicated that this insoluble carbonate was calcium carbonate (**7**). If this is the case, the experimental results obtained from the exchange reactions when substituted into the expression for the solubility product of calcium carbonate should yield a value for this constant which is in reasonable agreement with the values already reported. Inasmuch as the values obtained (table III) agree with the solubility product of calcium carbonate it may be assumed that the active substance is calcium carbonate.

The expression for the solubility constant of calcium carbonate is

$$\text{(B)} \qquad \frac{(Ca^{\cdot\cdot}) \cdot (HCO_3^-)^2 \cdot K''}{(H_2CO_3) \cdot K'} = K_{sp}$$

where the quantities in parentheses represent the activities of the components; K''/K' is the ratio of the secondary to the primary ionization constant of carbonic acid; and K_{sp} is the solubility product of calcium carbonate (**3**).

Assume that the concentration of the calcium ion is equal to the sum of the value *fr*, converted to moles per liter, plus the residual calcium ion present in sunflower-leaf sap (approx. 0.015 moles per liter). The total concentration of the calcium ion is shown in table III.

The bicarbonate ion was assumed to be equal to the sum of: the residual bicarbonate ion remaining fixed in the sap of the leaves (term *b*, equation A); the amount of carbon dioxide which reacted chemically, designated *c* (table II); and the amount brought into solution by dissolution of the insoluble calcium carbonate, *fr*. Algebraically this sum is $b + c + fr$, the values of which are given in table III.

The concentration of the dissolved carbon dioxide was calculated from the partial pressure of the carbon dioxide and its solubility in water at 15°, 0.0454 mol./kg./atm. (**2**).

At the ionic strength of sunflower-leaf sap, 0.2434 (**7**) the activity coefficient of calcium bicarbonate is 0.49 (**3**). The ratio K''/K' is 1/9100 (**3**).

By substitution of these values into equation B the following expression is obtained

$$\text{(C)} \qquad \frac{[0.49\ (fr + 0.015)]\ [0.49\ (b + c + fr)]^2}{9100\ (0.0454)\ P_{CO_2}} = K_{sp}$$

from which the values of K_{sp} reported in table III, were calculated. The

TABLE III

CALCULATION OF THE SOLUBILITY PRODUCT OF THE INSOLUBLE METAL CARBONATE, PRESUMABLY CALCIUM CARBONATE, FROM THE OBSERVATIONS ON ABSORPTION AND EXCHANGE OF CARBON DIOXIDE IN SUNFLOWER LEAVES

PRESSURE CO_2	*fr.* + Ca	BICARBONATE	CARBONIC ACID	K_{sp}
atm.	*M/L*	*M/L*	*M/L*	$\times 10^9$
0.262	0.02124	0.01578	0.01189	5.7
0.265	0.01858	0.01488	0.01203	4.4
0.287	0.02077	0.01582	0.01303	5.2
0.561	0.02529	0.02392	0.02547	7.3
0.612	0.02679	0.03032	0.02778	11.5
0.652	0.02429	0.02462	0.02960	6.4
0.714	0.02612	0.02836	0.03242	8.4
			Average	7.0

average value obtained for the solubility product is 7.0×10^{-9} at 15.0° C. This is in good agreement with the value obtained by FREAR and JOHNSTON which, extrapolated to 15° C., is 7.14×10^{-9}. From the agreement between the solubility product calculated from the data obtained from sunflower leaves and the solubility product found from physico-chemical measurement, it is apparent that calcium carbonate participates to an appreciable extent in the absorption of carbon dioxide by living sunflower leaves.

Additional evidence that the exchange in sunflower leaves may be almost completely accounted for by reaction of carbon dioxide with calcium carbonate is obtained from experiments in which leaf discs were saturated with radioactive carbon dioxide at about 5° C. and atmospheric pressure and then stored in air in the dark until all the free carbon dioxide had diffused out of the leaf. Referred to 10 gm. of leaf material, the amount of radioactive carbon dioxide retained, 2.49 ml., agreed well with the amount calculated from the solubility of calcium carbonate under the conditions obtained in the leaf, *viz.*, 2.65 ml. The amount of calcium carbonate potentially involved in the dissolution and exchange process corresponds to about 10.8 ml. of carbon dioxide. This is somewhat less than the total amount of carbon dioxide obtained from 10 gm. of sunflower leaves on acidification (table II) but this discrepancy may be due to incomplete mixing of the precipitated calcium carbonate with all of the calcium carbonate contained in the leaf.

By substitution of experimentally determined quantities in equation A, it is possible to calculate the amount of carbon dioxide absorbed by calcium carbonate in the leaf, *fr*. Subtraction of this quantity from the total amount of carbon dioxide absorbed chemically gives an estimate of the amount of carbon dioxide absorbed by buffer substances. The values so calculated were found to be:

P_{CO_2}	0.262	0.265	0.287	0.561	0.612	0.652	0.714	atm.
Buffer abs.	0.39	1.17	0.56	0.40	1.00	0.88	0.89	ml.

In general the amount of carbon dioxide absorbed by buffers increased with increase in the partial pressure of the carbon dioxide. So far the nature of the buffer system has not been determined completely.

The pH of sunflower-leaf sap

From the data at hand it is possible to estimate the pH of the sap within the sunflower leaf. The ionization constant of carbonic acid has been reported in a previous paper, where the expression relating it to the pH of sunflower-leaf sap, expressed from the leaf, has been given. The equation was found to be:

$$\text{(D)} \qquad pH = 6.425 + 1.343 - 0.177 - pHCO_3 + pP_{CO_2}$$

(1.343, – logarithm of the solubility of carbon dioxide; 0.177, – logarithm of the activity coefficient of bicarbonate ion; and 6. 425, – logarithm of the ionization constant of carbonic acid). As is shown in table III, the total bicarbonate ion concentration in the sunflower leaf is known at various partial pressures of carbon dioxide. By substituting the appropriate functions of the bicarbonate ion concentrations and of the partial pressures of the carbon dioxide in equation D the values of the pH of the sunflower-leaf sap have been calculated.

P_{CO_2}	0.262	0.265	0.287	0.561	0.612	0.652	0.714
pH calc.	6.37	6.34	6.33	6.22	6.29	6.17	6.19
pH sap.	6.35	6.34	6.32	6.10	6.07	6.05	6.02

Comparison of these values with the values obtained by direct measurement of expressed sap, obtained in the previous investigation (**7**) and interpolated to the same partial pressure of carbon dioxide, shows that reasonable agreement exists between the two results, and that the intact leaf is a better buffer than the expressed sap.

Assimilation of carbon dioxide by unilluminated leaves

Unilluminated leaves combine chemically with carbon dioxide. This has been demonstrated by observing a "dark pick-up" of carbon dioxide immediately following photosynthesis by wheat leaves (**4**) and also by observing the carbon dioxide absorption by leaves from a number of species of plants under equilibrium conditions of carbon dioxide pressure (**7**). In sunflower leaves this chemical absorption is accomplished largely by reaction with buffers and insoluble carbonates, presumably calcium carbonate. Besides this, however, another type of absorption takes place in the dark in which carbon dioxide becomes bound much more firmly than in carbonates. Our experiments, as well as the earlier ones of Ruben, Kamen, and their associates (**5, 6**) have demonstrated this by the use of radioactive carbon dioxide. This absorption has been attributed to the formation of organic compounds which supposedly contain the radioactive carbon in carboxyl groups (**6**). Our experiments have given no definite knowledge concerning the nature of the compound formed by this reaction.

The leaf discs (4.5 cm. diameter) to be treated with radioactive carbon dioxide were placed in a test tube, evacuated with a Hyvac pump, and saturated with radioactive carbon dioxide at atmospheric pressure and at about 5° C. The leaf discs were freed from all carbonates by acidification with 2 ml. of 6 N hydrochloric acid and evacuation with an aspirator pump, warming the mixture in a water bath during the evacuation. All manipulations previous to the acidification were carried out in the dark.

The radioactivity of the leaf residue and acid solution was determined. Any activity observed was ascribed to noncarbonate, radioactive carbon.

The amount of noncarbonate, radioactive material usually formed in 10 gm. of fresh sunflower leaves by one treatment with radioactive carbon dioxide corresponded to absorption of approximately 0.304 ml. of carbon dioxide. The product formed is completely soluble in water. The radioactive carbon is bound so that it cannot be freed by hydrolyzing the material for a few minutes with either warm acid or alkali.

Only traces of this material were formed by leaves killed by freezing previous to the absorption of radioactive carbon dioxide, although freezing

subsequent to absorption did not destroy the material formed. It is probable that killing destroys the ability of leaves to form this material.

The amount of radioactive, noncarbonate material does not increase with time of contact between leaves and radioactive carbon dioxide, at least after fifteen minutes, as the following experiment shows: Four samples of sunflower leaves were kept in an atmosphere of radioactive carbon dioxide for varying lengths of time. These samples were removed from the radioactive gas at the time intervals indicated and were tested for radioactivity after acidification. From the data obtained it is apparent that no significant increase in the amount of noncarbonate compounds was observed after the first fifteen minutes. How short a time is necessary to reach the maximum concentration has not yet been determined.

Interval of storage (minutes)	15	29	44	59
Logarithm of activity referred to common time	1.70 1.63	1.76 1.7(	1.71 1.68	1.74 1.76

In a single experiment when the leaf was saturated with ordinary carbon dioxide before evacuation and exposure to an atmosphere in radioactive carbon dioxide, less than the average amount of radioactive carbon dioxide was fixed: average amount fixed, including several determinations not listed in table IV, 0.0393 ml.; amount fixed after pre-treatment with ordinary carbon dioxide, 0.0198 ml. (table IV).

There is indication that in the living leaf exchange takes place between ordinary carbon dioxide and the noncarbonate radioactive carbon fixed in the dark. In order to prove this conclusively, however, more critical experiments need to be performed (table IV, note e).

Multiple saturation with radioactive carbon dioxide increased the amount of noncarbonate radioactive compound. In one experiment when three discs of leaves (approx. 1.29 gm.) were evacuated and saturated three times with radioactive carbon dioxide; 0.0841 ml. of carbon dioxide was fixed as noncarbonate carbon whereas the average amount fixed by a single saturation was 0.0393 ml. (table IV).

Three types of exchange reaction suggest themselves: (1) a statistical exchange with a compound completely dissolved in the sap of the leaf; (2) an exchange depending on the dissolution of some material by increased partial pressures of carbon dioxide; and (3) an exchange brought about by alternate dissociation and formation of some compound caused by alternate evacuation and saturation with carbon dioxide.

Analysis of these three possibilities, taking into account the data obtained by multiple saturation with radioactive carbon dioxide and also the solubility of carbon dioxide in the sunflower leaf, makes it seem unlikely that a statistical exchange takes place with a compound completely dissolved

in the sap of the leaf. The other two possibilities appear to be much more reasonable. A choice between these two cannot be made from the data available at the present time.

Tentative estimates of the amount of compound potentially capable of exchanging carbon dioxide in this way yield a value of from 0.004 to 0.005 moles per kilogram of fresh sunflower leaves. In this calculation it was assumed that the reaction takes place between equimolecular quantities of the two reactants.

TABLE IV

COMPARISON OF CARBONATE AND NONCARBONATE FIXATION OF CARBON DIOXIDE BY UNILLUMINATED SUNFLOWER LEAVES
VOLUMES OF CARBON DIOXIDE

INITIALLY ABSORBED	RETAINED ON STORAGE	RETAINED ON ACIDIFICATION
ml.	*ml.*	*ml.*
1.112	.....	0.0302
0.793	.....	0.0367
0.983*	.....	0.0198
1.205	0.329†	.....
.....	0.146‡	0.0168
1.807§	.....	0.0841
0.546	0.246	0.0178
0.679	0.322	0.0298
2.733	0.388	0.0226
1.138	0.361	0.0294
1.045	0.463	0.0656

* Initially pretreated with ordinary carbon dioxide.
† Pretreated initially with ordinary carbon dioxide and then saturated with radioactive carbon dioxide, evacuated and swept out once with air.
‡ Same as † but swept out an additional three times with ordinary carbon dioxide.
§ Saturated three times with radioactive carbon dioxide.

In table IV a comparison is made of the amount of radioactive carbon, in terms of carbon dioxide, retained by sunflower leaves after storage and after acidification. The amount retained after acidification (noncarbonate carbon) is roughly 10 per cent. of the carbonate carbon. The amount of carbonate carbon dioxide may be obtained by subtracting the values listed in column 3 from the corresponding values in column 2. The amount of radioactive carbon dioxide measured initially is of little significance because of the differences in the amount of diffusion of the gas from leaves occasioned by the transfer of the leaves to the measuring vessel.

PHOTOSYNTHESIS

One of the chief purposes for investigating the absorption of carbon dioxide by leaves is to determine how carbon dioxide absorbed from the air becomes involved in the photochemical reaction of the photosynthetic process. By the use of radioactive carbon dioxide it has been possible to demonstrate

that leaves can utilize for photosynthesis carbon dioxide which has already been absorbed by the leaves. In this way it has been shown that the absorption of carbon dioxide by leaves for photosynthetic use is independent of the photochemical reaction. Since the photosynthesis of carbon dioxide already contained within the leaf can be followed, and since it is possible to differentiate between different types of combination of carbon dioxide within the leaf, it may be possible to determine which fractions of the carbon dioxide are used in the photosynthetic process.

Sunflower leaves (discs 4.5 cm. diameter) were saturated in the dark with radioactive carbon dioxide at one atmosphere pressure and about 5° C. The discs were removed from the radioactive carbon dioxide and as quickly as possible brought into sunlight. After fifteen minutes of insolation the discs were acidified with hydrochloric acid and the liberated carbon dioxide removed by warming in a vacuum. The radioactivity of the acidified leaf residue and the solution from the insolated leaves was in two experiments 1.49 and 3.03 times the radioactivity of the noninsolated leaves.

Evacuation of leaves after they had been saturated with radioactive carbon dioxide reduced the amount of radioactive carbon fixed by illumination. In one experiment the reduction was from 3.14 to 1.35 arbitrary units. (The units used were the ratios of the radioactivities of the illuminated to unilluminated leaves, measured after acidification.)

Leaves depleted of their easily removable carbon dioxide, by permitting this fraction to diffuse into the atmosphere in the dark, also showed decreased photosynthesis. The amount of radioactive carbon fixed by illumination was less, the longer the time the leaves were stored in the dark (table V).

TABLE V

EFFECT OF CARBON DIOXIDE DIFFUSION ON THE AMOUNT OF PHOTOSYNTHESIS

Interval in dark	Carbon dioxide contained		Noncabronate carbon fixed		Illumination
	Total	Mobile	Total	Photosynthesis	
min.	*ml.*	*ml.*	*ml.*	*ml.*	*min.*
0.0	0.363	0.298	0.0397	0.0370	81
16.7	0.156	0.0911	0.0141	0.0114	25
30.2	0.0981	0.0337	0.0119	0.0091	25
109.8	0.0644	0.000	0.0027	0.000	0
0.0	0.468	0.348	0.0245	0.0147	15
19.0	0.149	0.029	0.0152	0.0054	15
59.4	0.120	0.000	0.0098	0.000	0
0.0	0.620	0.464	0.0392	0.0173	15
17.1	0.173	0.019	0.0247	0.0028	15
69.7	0.154	0.000	0.0219	0.000	0

From these experiments it is apparent that removal of the loosely bound carbon dioxide decreases the amount of photosynthesis, although it is not clear whether the decrease is due to loss of free carbon dioxide or to a diminution of some easily dissociable compound.

RATE OF PHOTOSYNTHESIS.—Rate measurements of the photosynthesis of radioactive carbon dioxide showed that most of the fixation occurred in the first few minutes of illumination (fig. 1). This is not surprising in view of

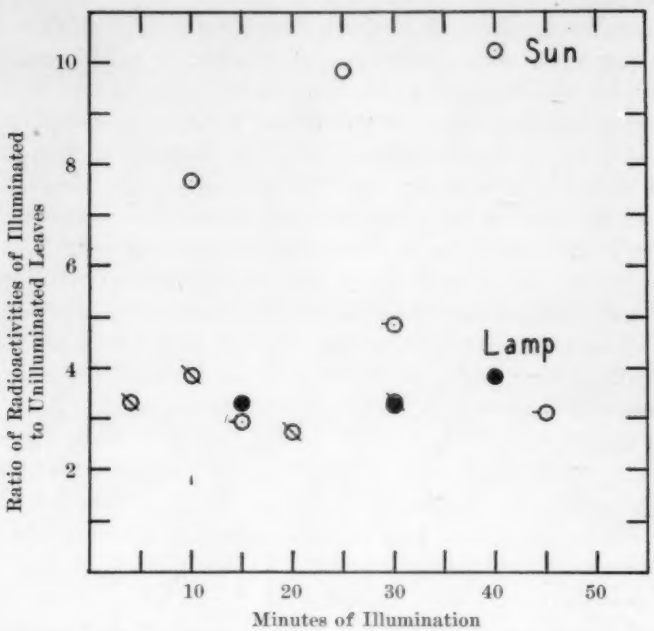

FIG. 1. Rate of photosynthesis of radioactive carbon dioxide contained within the leaf.

Experimental points marked alike belong to the same series of measurements. The points marked with an open circle, ○, were obtained by solar illumination; all other points were obtained by illumination with a 500-watt Mazda lamp.

the dependence of photosynthesis on the content of radioactive carbon dioxide within the leaf. As may be seen from table V this amount decreases very rapidly with time. The rate measurements were made in the following manner.

Discs cut from sunflower leaves (4.5 cm. in diameter) were saturated with radioactive carbon dioxide and then illuminated. The discs were placed on blotting paper which was saturated with water and laid in a brass pan. This pan was set on four supports in another pan of water; this arrangement retarded the loss of water from, and excessive heating of, the

leaves. A glass shield was supported over the leaves in order to leave a considerable space for air circulation. Another glass plate mounted on four tall legs covered the whole assembly. Air blown through the assembly cut down the heating effect and quickly removed any radioactive carbon dioxide which diffused out of the leaves. The results of these experiments are shown in figure 1 where the ordinates are the ratios of the radioactivities of the illuminated to the unilluminated leaves, determined after acidification.

Too much significance must not be attached to the enhanced photosynthesis observed for sunlight as compared to light from a 500-watt Mazda lamp; factors other than increased light intensity were probably involved, as other experiments indicated.

In these experiments the leaves were not deprived of atmospheric carbon dioxide, and it is possible that this carbon dioxide reacted with insoluble radioactive carbonates thus making available radioactive carbon dioxide for photosynthesis. Whether this type of absorption and exchange is operative can be easily determined by future experiments.

The fact that the photosynthetic process can utilize carbon dioxide that has been stored in the leaf for some time must be taken into account when interpretations are made of measurements on such quantities as photosynthetic quotient, quantum yield, and induction periods.

Respiration of newly formed photosynthate

Experiments were carried out to determine whether the carbon newly assimilated in photosynthesis was easily lost through respiration. Discs of sunflower leaves were saturated with radioactive carbon dioxide and then illuminated for a short period of time. The discs were placed in the dark and tested at various intervals for the amount of noncarbonate radioactive carbon they contained. The ratio of this amount to the amount contained by an unilluminated leaf is plotted against time in figure 2. The results show that the loss due to respiration is quite rapid. Whether this rate is greater or less than the rate of loss of carbon from other organic compounds contained in the leaf, *e.g.*, glucose, was not determined.

Summary

1. The investigation of the absorption of carbon dioxide by living sunflower leaves by means of radioactive carbon dioxide has shown that the three possible methods of absorption, previously proposed, are all operative; namely, solution in the water of the sap, reaction with soluble buffer substances, and reaction with insoluble carbonates, presumably calcium carbonate.

2. In addition to these reactions, carbon dioxide reacts to form a noncarbonate derivative of which little is known.

3. The carbon dioxide absorbed previous to illumination can be used in the photosynthetic process. From this it is evident that the absorption of carbon dioxide for photosynthetic purposes is not a part of the photochemical reaction.

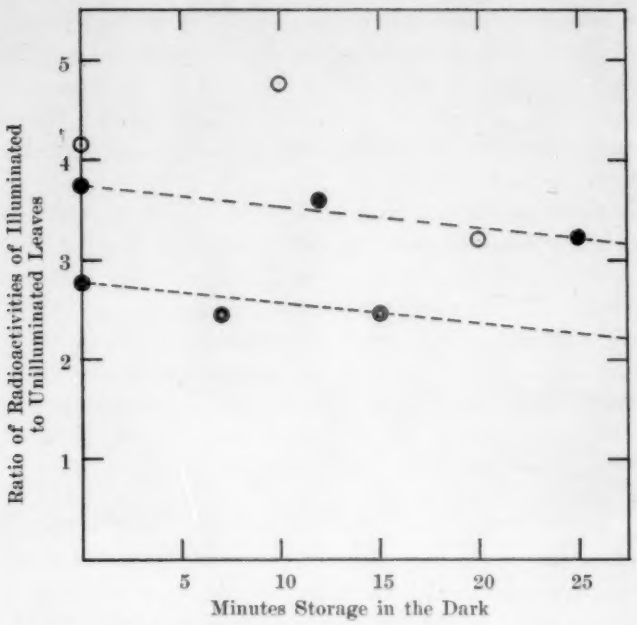

FIG. 2. Rate of respiration of newly formed photosynthate.
Experimental points marked alike belong to the same series of measurements.

4. The newly formed photosynthate is quite rapidly used up in respiration. How its rate of respiration compares with the rate of respiration of other organic compounds con ained within the leaf has not been determined.

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NATURE OF BOUND WATER IN COLLOIDAL SYSTEMS

RAY CALVIN CHANDLER

(WITH ONE FIGURE)

Introduction

The state of water in colloidal systems is a controversial subject. Discovery of the colloidal state by GRAHAM (**18**) was followed by extensive studies by VAN BEMMELN (**66, 67, 68, 69**) and ZSIGMONDY (**73, 74**). Thermodynamic studies by KATZ (**36**) indicated significant changes in free energy and heat content in colloidal systems at low water content. OVERTON (**51**) concluded that part of the water in animal tissue did not have normal solvent properties. The work of ROSA (**59**) and HOOKER (**28**) suggested a relationship between certain hydrophilic bio-colloids and cold resistance in plant tissue. NEWTON and GORTNER (**49**) proposed a cryoscopic method for the measurement of hydrophilic colloids as a criterion of the state of water in living and non-living colloid systems. The apparently unusual state of water thus disclosed by a vast amount of data has been called "bound" water. It has been assumed that the presence of colloidal material was responsible for the observed phenomenon. It is of particular importance in biological systems because of certain implications with regard to hardiness and drought resistance in plants and animals. The majority of investigations have dealt with plant physiology.

The estimation of bound water is based upon the physical measurement of a variety of properties of water. Some workers compute the amount of bound water from the total change in thermodynamic activity of the water in a given system while other workers include only that part of the change in thermodynamic activity which cannot be accounted for by osmotically active substances. In this paper the calculations are based upon the latter concept and bound water is defined as the amount of water required to compensate for the deviation of some thermodynamic property of water from the measurement expected in simple solution.

It has been suggested (**17**) that bound water is due to the orientation of water molecules about the colloidal particles and that the water, thus removed from the body of the solution, loses its solvent properties. A few investigators have disagreed with these premises. The data of HILL (**27**) and GROLLMAN (**21**) did not show appreciable amounts of bound water in many colloidal systems. GREENBERG and GREENBERG (**20**) subjected the concept of lost solvent powers to test and found no evidence for bound water on that basis. A knowledge of the nature of the phenomenon observed is necessary for the interpretation of its rôle in biological processes.

Methods for bound water studies

The methods used to measure the properties of water are based on physico-chemical laws applicable to all components of the solution. Attention is centered upon water since it is the major constituent and lends itself readily to measurement. Perhaps the most frequently used methods are based upon the colligative properties of the solution, *i.e.*, vapor pressure lowering, freezing point depression, and osmotic pressure which are interrelated through the applicability of the gas laws to solutions. The chief methods used for the determination of bound water may be classified on the following basis:

I. Colligative measurements.
   (a) Freezing point depression when solutes are added (**3, 49, 60, 63, 64**)
   (b) Vapor pressure methods (**2, 15, 16**)
   (c) Osmotic pressure (**40, 61**)

II. Change of state.
   (a) Latent heat of fusion (**45, 57, 60, 65**)
   (b) Volume change (**32, 43, 59, 60**)
   (c) Heat of vaporization (**21, 27**)

III. Distribution of solutes.
   (a) Ultrafiltration (**20**)
   (b) Differential concentration (**44**)

IV. Resistance to pressure (**45, 48, 63**).

Special methods other than the above have been applied to the study of this problem (**1, 15, 22, 23, 29, 42**).

Interpretation of colligative measurements is based on limiting laws for ideal solutions such as might be found in dilute binary solution. The assumption that the thermodynamic contribution of several components in a complex solution is additive is false. The heat of vaporization method introduced by Hill (**27**) directly compared complex solutions and largely obviated this assumption. The difficulties to be met in these studies may be illustrated by the work of Kistler (**38**) and Dexter (**10**). The former concluded that all methods depending upon freezing may involve errors due to non-equilibrium and the latter found irreversibility of ice formation in bio-colloids. Some methods are well suited to a study of the properties of water at high water content while other methods are better suited to systems at low water content.

The problem

This investigation was planned as a study of true and colloidal solutions to disclose the factors responsible for deviations in colligative measurements which are often employed in the estimation of bound water. Any given

colligative determination has a theoretical value based on the ideal gas laws but usually its empirical value differs somewhat from the theoretical. The calculations in this paper are based on the empirical value for a substance dissolved in pure water. A measurement which yields a value greater than expected is called a *positive* deviation while a measurement less than the empirical value is called *negative*. By appropriate sequence of experiments both positive and negative deviations are produced and an attempt is made to evaluate the factors responsible for the deviations.

Methods and materials

Two colligative methods were used in this study on the state of water. The first consisted of vapor pressure measurements made by a highly sensitive static vapor pressure apparatus described in a previous paper (**5**). Gelatin was selected as a typical hydrophilic colloid upon which much data has been secured (**3, 16, 20, 21, 22, 23, 29, 32, 35, 42, 46, 50, 58, 65**). Potassium chloride was selected as a reference solute since its vapor pressure in true solution has been shown to behave in a regular manner over the range of concentrations to be studied (**14**); furthermore its combination with gelatin (**50**) has been found to be negligible.

The second method utilized freezing point data obtained by use of Beckmann thermometer, freezing point tube, air chamber and insulated ice bath. Glucose and KCl were selected as reference solutes. The thermometer was calibrated and kept at low temperature while not in use. Molal freezing point depression-concentration curves were prepared from data (**31**) for each reference substance. The various electrolyte, non-electrolyte, and dipolar solutions were prepared from C.P. standard chemicals. Eastman's de-ashed gelatin was used. The plant sap examined was expressed from plants that had been "hardened" by subjection to low temperature for a period of time.

This application of the cryoscopic method for the determination of bound water differs from the usual method in using two types of solutes (electrolyte and non-electrolyte) as reference substances instead of confining the measurements exclusively to one reference substance.

Theoretical considerations

Vapor pressure constitutes an ideal method for calculation of thermodynamic functions in the study of solutions. In an attenuated atmosphere, as under its own vapor pressure, the vapor of an aqueous solution may be considered as a perfect gas in rapid reversible equilibrium with the liquid phase. The vapor pressure (p) is proportional to the fugacity (f)[1] and the activity (a)[2] is equal to the relative vapor pressure (p/p°). At constant

[1] **Fugacity**: the vapor pressure of a gas which obeys the ideal gas law.

volume, as under condition of these experiments the free energy (F)[3] and the vapor pressure are related by the equation

$$\triangle F = RT \ln p/p^{\circ} \qquad 1$$

which represents the free energy change involved in the isothermal reversible transfer of one mole pure water to the solution. The differential rate of change of a property with addition of a substance to the solution is called a partial molal quantity. Assuming that the partial molal heat content ($\overline{H}$)[4] is constant over the temperature range studied, its value may be calculated from the relationship

$$\overline{H} = -\frac{RT^2 \, d \ln p^{\circ}/p}{dT}. \qquad 2$$

The change in heat content calculated by this method presumably differentiates between kinetic energy which is a function of temperature and the orienting or binding forces which are independent of temperature. The partial molal entropy ($\overline{S}$)[5] of the water component may be obtained from the relationship

$$\overline{S} = \frac{\overline{H} - \overline{F}}{T}. \qquad 3$$

KATZ (**37**) considers entropy to be a measure of the change in randomness of water molecules in colloidal systems.

The phenomenon of solution is a reflection of interacting forces between components and no one component may be singled out arbitrarily as the cause of the deviations. The properties of the solution are determined by the similarities and dissimilarities of all components. Ideal solutions are rare. The vast majority show deviations from ideal behavior even in binary solution. The energy relations between components of any solution is expressed by the equation (**41**)

$$\frac{N_1 \, (d \ln f_1)}{dN_1} + \frac{N_2 \, (d \ln f_2)}{dN_1} + \frac{N_3 \, (d \ln f_3)}{dN_1} \ldots = 0 \qquad 4$$

where N is the mole fraction, f the fugacity, subscripts indicate components; temperature, pressure, and volume are assumed constant. Activity or vapor

[2] Activity: the thermodynamically effective concentration of a constituent rather than the molar concentration.

[3] Free energy: the energy change in a process which may be used to do work; a measure of the tendency of a reaction to go in a given direction. Under conditions of these experiments it equaled the maximum work possible. It also equaled the total energy change less the amount involved in entropy change.

[4] Heat content: the total energy content, in calories, of a gram mole of a constituent. Actually only the change in heat content can be measured. The gain or loss is due to changes in physical properties of the molecule.

[5] Entropy: the energy, in calories per degree, absorbed in a process but not available for work. As used here positive values indicate a more random condition, negative values a less random condition of the molecules.

pressure may be substituted for fugacity in this equation which emphasizes the fact of energy exchange within the solution.

The possibilities of deviations in actual solutions containing ions, non-electrolytes, and dipolar compounds of diverse electrical properties and molecular sizes may well be considered. In a typically non-ideal solution of ions the DEBYE-HÜCKEL theory (**7, 30**) indicates that under constant conditions of dielectric constant and temperature the activity of the ions is affected by a number of factors. This is shown by the equation

$$-\ln \gamma \text{ (ions)} = \frac{N^\circ z^2 e^2 b\sqrt{\mu}}{2\,DRT\,(1-ab\sqrt{\mu})} \qquad 5$$

where γ is the activity coefficient; N° the Avagadro number; z, valence; e, unit charge; D, dielectric constant; T, absolute temperature; μ, a function of concentration and valence; a, ion diameter; while R and b are constants. DEBYE and MCAULAY (**9**) and SCATCHARD (**62**) have applied the concept to non-electrolytes in ionic solutions and found the relation.

$$-\ln \gamma \text{ (non-electrolyte)} = -\frac{N^\circ e^2 \mu \alpha}{DRT\,c} \qquad 6$$

where α, a constant characteristic of the non-electrolyte, has a positive value when the non-electrolyte reduces the value of the dielectric constant, and c indicates the radius of the particle. Equations 5 and 6 show that (in a common environment) concentration, valence, dielectric constant, and temperature have opposite effects upon the activities of electrolytes and non-electrolytes in many cases.

In a two-component solution according to equation 4 $N_1 \frac{(d \ln a_1)}{dN_1} = -N_2 \frac{(d \ln a_2)}{dN_1}$; that is, a change in activity of one component indicates an opposite change in activity of the other component.

In a three component solution which is saturated with respect to the second component, the activity of the second component is fixed and under such conditions $N_1 \frac{(d \ln a_1)}{dN_1} = -N_3 \frac{(d \ln a_3)}{dN_1}$; that is, the change in activity of the solvent reflects a change in activity of the third component. Thus the vapor pressure of an aqueous solution may be utilized to determine energy relations between components, to measure the degree of orientation of water molecules, and to study fundamental differences between kinds of solutes.

Experimentation

The experimental work may be divided into the four following steps:

(1) A vapor pressure study on a two component true solution;

(2) A vapor pressure study on a two component colloidal solution;

(3) A vapor pressure study on a three component colloidal solution;
(4) Freezing point studies on true and colloidal solutions.

Vapor pressure measurements were made on a dilute potassium chloride solution at two temperatures to determine thermodynamic functions of the water component. The data appear in table I and energy data are listed

TABLE I

VAPOR PRESSURE LOWERING IN A DILUTE KCl SOLUTION (0.109 M) AT 1° C. AND 20° C.

	H_G, 20° C.	H_G, 1° C.
	mm.	*mm.*
	0.0614	0.0162
	0.0616	0.0160
	0.0612	0.0159
Mean	0.0614	0.0160

in table IV. Consideration of the data in table IV shows that the heat content and entropy of the water was slightly greater than for pure water.

The second experimental solution was an aqueous solution of isoelectric gelatin (3.45 per cent.). It was subjected to vapor pressure measurements at the same two temperatures. The data are listed in table II and values for the thermodynamic functions appear in table IV. A very slight increase in heat content and entropy of the water component is indicated by these data, suggesting that in spite of the lowering of vapor pressure and decreased activity of water in this solution the water molecules actually have slightly greater freedom for random distribution. In accordance with equation 4, the decrease in activity of the water component reflects an increase in activity of the gelatin component, regardless of the fact that its mole fraction is unknown.

TABLE II

VAPOR PRESSURE LOWERING OF A 3.45 PER CENT. GELATIN SOLUTION

	H_G, 20° C.	H_G, 1° C.
	mm.	*mm.*
	0.0013	0.0003
	0.0015	0.0003
	0.0022	0.0003
	0.0018	0.0002
Mean	0.0017	0.0003

The third solution to be investigated was a three component colloidal solution consisting of the previously discussed isoelectric gelatin solution to which was introduced a gas-free charge of KCl producing a 0.069 M solu-

tion with respect to KCl. Vapor pressure data for this solution are tabulated in table III. The vapor pressure lowering due to gelatin is assumed to be constant in solutions 2 and 3. The vapor pressure lowering due to KCl is found by difference. The observed molal vapor pressure lowering $\frac{(P^\circ - P)}{M}$ for KCl in the colloidal solution is compared to that determined from a study of solution 1. Negative deviations are found which also show a marked temperature coefficient. The partial molal functions for water given in table IV show that for this colloidal solution, as in the previous one, the heat content and entropy of the water has been increased slightly by the presence of the colloidal substance. Assuming that the gelatin solutions are saturated with respect to gelatin, the activity of gelatin is unchanged in solutions 2 and 3, therefore the change in molal vapor pressure lowering of KCl shown in table III indicates a change in activity

TABLE III

VAPOR PRESSURE LOWERING OF 0.069 M KCl SOLUTION IN PRESENCE OF 3.45 PER CENT. GELATIN

	Hg, 20° C.	Hg, 1° C.
	mm.	*mm.*
	0.0384	0.0093
	0.0398	0.0097
	0.0387	0.0093
	0.0406	0.0092
	0.0393	0.0090
	0.0379	0.0093
	0.0382	
	0.0386	
	0.0395	
Mean	0.0390	0.0093
VPL due gelatin	0.0017	0.0003
VPL due KCl	0.0373	0.0090
$\frac{P^\circ - P}{M} = \frac{0.0373}{0.0693} =$	0.5382	$\frac{0.0090}{0.0693} = 0.1299$
$\frac{P^\circ - P}{M}$ (KCl − H_2O) (system) =	0.5837	0.1540
Ratio $\frac{\text{observed}}{\text{empirical}} =$	0.922	0.844
Percentage deviation	−7.8	−15.6

of KCl as well as water. In each solution the heat content and entropy of the water component is slightly greater than for pure water. Assuming that entropy is a measure of randomness of water molecules it appears that the water molecules are quite as free in any of these solutions as in the pure

solvent. The observed deviation, therefore, is not due to the orientation of water molecules but to the interaction of the components of the solution.

TABLE IV

PARTIAL MOLAL FUNCTIONS OF WATER COMPONENT IN DILUTE COLLOIDAL AND NON-COLLOIDAL SOLUTIONS

SYSTEM	$\overline{F_1}$°c.	$\overline{H_1}$°$_{-20}$°c.	$\overline{S_1}$°c.
(1) H_2O–KCl (0.109 M)	−1.78	1.74	0.013
(2) H_2O–gelatin (3.45%)	−0.03	0.48	0.002
(3) H_2O–gelatin–0.069 M KCl	−1.00	2.52	0.013

The fourth line of evidence dealt with freezing point studies on both colloidal and non-colloidal solutions of varying complexity. The first series were three-component solutions consisting of water and two reference solutes used in these experiments. The data are shown in table V. The calculated molal freezing point depression refers to the value obtained when the solutes are dissolved in pure water.

TABLE V

DEVIATIONS IN FREEZING POINT DEPRESSION WHEN GLUCOSE IS ADDED TO NON-COLLOIDAL SOLUTIONS CONTAINING IONS

SYSTEM	SOLUTE ADDED	MOLAL FREEZING POINT DEPRESSION		DEVIATION
		CALC.	OBS.	
	mols.	°*C.*	°*C.*	%
(a) H_2O–0.21 M KCl	0.40 glucose	1.89	1.98	4.8
(b) H_2O–0.42 M KCl–0.40 M glucose	0.40 glucose	1.91	2.12	11.0

When the non-electrolyte glucose was placed in the non-colloidal solution containing ions the freezing point depressions were greater than calculated values and the magnitude of the positive deviations was a function of the ion concentration.

A second type of non-colloidal solution was next examined, using glucose as the reference solute. Aqueous solutions of amino acids are dipolar in nature and constitute important constituents of many natural solutions. When the non-electrolyte was added to these compounds the magnitude and sign of the deviations varied considerably and was apparently a function of length of the carbon chain. The data are given in table VI. The effect of ionic solutions, including KCl, upon the freezing point depression of amino acids may be found in table VIII.

TABLE VI

DEVIATIONS IN FREEZING POINT DEPRESSION WHEN GLUCOSE IS ADDED TO AMINO ACID SOLUTIONS (DIPOLAR COMPOUNDS)

AMINO ACID	CARBON ATOMS	GLUCOSE	MOLAL FREEZING POINT DEPRESSION		DEVIATION
			CALC.	OBS.	
		mols.	°C.	°C.	%
0.5 M glycine	2	0.74	1.906	1.861	−2.4
0.5 M alanine	3	0.75	1.907	1.916	0.5
0.5 M valine	5	0.73	1.906	2.000	4.9

The third group examined contained colloids. The positive deviations noted for glucose in the presence of simple solutions of gelatin and hemoglobin (table VII, *a* and *c*) are characteristic of the data usually reported for bound water. The tendency of KCl to give negative deviations in many complex solutions is striking. A sample of plant sap known to contain natural dipolar and ionic compounds yielded a large positive deviation when

TABLE VII

DEVIATION IN FREEZING POINT DEPRESSIONS AS VARIOUS COMPONENTS ARE ADDED TO SOLUTIONS

SYSTEM	SOLUTE	MOLAL FREEZING POINT DEPRESSION		DEVIATION
		CALC.	OBS.	
	mols.	°C.	°C.	%
(a) H_2O–5% gelatin	0.80 glucose	1.910	2.00	4.7
(b) H_2O–5% gelatin–0.8 M glucose	0.40 KCl	3.315	3.28	−1.1
(c) H_2O–5% hemoglobin	0.69 glucose	1.905	2.07	8.6
(d) H_2O–5% hemoglobin–0.4 M glucose	0.22 KCl	3.375	3.24	−4.0
(e) Wheat sap ("hardened")	0.44 glucose	1.895	2.18	15.1
(f) Wheat sap ("hardened")	0.31 KCl	3.360	3.08	−8.3
(g) H_2O–0.4 M KCl–0.8 M glucose	5% gelatin	1.860	(−0.037)*	.....
(h) H_2O–0.5 M alanine–0.5 M glucose	5% gelatin	1.860	(−0.015)*	.....
(i) H_2O–0.5 M glycine–0.74 M glucose	5% gelatin	1.860	(−0.013)*	.....

* Observed elevation of freezing point which *rose* slightly when gelatin was added.

glucose was used as the reference solute but a negative deviation when KCl was the reference solute.

The foregoing experiments roughly differentiate the effects of typical substances; namely, electrolytes, non-polar and dipolar non-electrolytes, and hydrophilic colloids. Some, and often all, of these substances are components of systems examined for bound water. Each of these might be expected to play a part in this problem owing to structure or electrical properties.

TABLE VIII

INFLUENCE OF ENVIRONMENT IN NON-COLLOIDAL COMPLEX SOLUTIONS UPON THE "NORMAL" BEHAVIOR OF COMPONENTS

GROUP	SOLVENT	SOLUTE ADDED	DEVIATION	METHOD	REFERENCE
	mols.		%		
(A)	0.140 KCl	0.372 M sucrose	5.8	Freezing point	(9)
	0.236 "	0.611 " "	9.5	" "	"
	0.369 "	0.851 " "	15.7	" "	"
	0.467 "	0.756 " "	18.5	" "	"
	0.703 "	0.511 " "	26.9	" "	"
	0.941 "	0.263 " "	34.9	" "	"
(B)	0.335 LiCl	Succinic acid	−12.6	Solubility	(39)
	0.40 NaCl	" "	− 8.4	"	"
	0.67 LiCl	Boric acid	−13.2	"	"
	0.80 NaCl	" "	− 1.5	"	"
(C)	0.50 LiCl	Glycine	8.0	"	(53)
	0.50 NaCl	"	3.9	"	"
	0.50 $NaNO_3$	"	12.2	"	"
(D)	0.50 KCl	Glycine	0.9	"	"
	2.00 KCl	"	− 9.2	"	"
(E)	0.50 NaCl	Leucine	− 9.5	"	"
	0.50 KCl	"	− 8.1	"	"
(F)	0.50 $BaCl_2$	Leucine	13.9	"	"
	0.50 $CaCl_2$	"	17.6	"	"
(G)	0.50 NaCl	Glycine	−14.0	Freezing point	(52)
	0.50 NaCl	Alanine	− 3.0	" "	"
(H)	0.50 $BaCl_2$	Glycine	−28.0	" "	"
	0.50 $BaCl_2$	Alanine	−15.0	" "	"
(I)	0.50 CH_3COOK	Glycine	− 5.2	" "	"
(J)	0.10 KNO_3	Glycine	− 6.8	" "	"
	0.50 KNO_3	"	−22.4	" "	"
(K)	0.01 $ZnCl_2$	Glycine	− 2.6	Potentiometric	(34)
	0.10 "	"	−19.4	"	"
	0.10 "	Valine	− 9.1	"	"
	0.50 "	"	−42.2	"	"

Discussion

The assumption that anomalous changes in activity of a substance in solution are based on chemical combination, association, or dissociation has been convenient; the concept of forces other than these, however, should not be neglected. From a thermodynamic standpoint any change in free energy must be balanced by an equal and opposite change within the system. Vapor pressure measurements have been utilized in this work on relatively simple solutions because of the high accuracy obtainable for very dilute solutions where concentration effects were at a minimum. Utilizing thermodynamic concepts it appears that the activity of water may deviate from

usual values to either greater or less values without materially affecting the randomness of orientation of water molecules.

The negative deviation of vapor pressure lowering of KCl solution in the presence of gelatin indicated that the activity of KCl had been decreased. If this is a fundamental principle it would be reflected in the solubility of a slightly soluble electrolyte in the presence of a colloid. FAILEY (**12**) found the solubility of TlCl in the presence of edestin agreed with that concept. JOSEPH (**35**) also found a decrease of activity of many salts in the presence of gelatin. GORTNER and GORTNER (**17**) and GERIKE (**16**) present data subject to the same interpretation. It seems evident that the changes taking place in the solution as indicated by a measurement of the water are the result of the interaction of all components and not the effect of the colloid alone.

The increase in heat content of colloidal solutions as indicated in the data of table IV is opposed to the concept of "binding" or molecular orientation as a dominant factor in the solution. One should expect orientation to decrease the heat content. The data presented here are supported by the findings of HAMPTON and MENNIE (**22**) and HORN and MENNIE (**29**) for gelatin solutions.

The deviation from additivity of colligative properties in colloidal solutions, which is sometimes used as a measure of the effect of hydrophilic colloids on the state of water therein, may be produced also in non-colloidal solutions as shown in tables V, VI, and VIII. By use of the non-electrolyte in the presence of ions large positive deviations may be produced. By use of a suitable electrolyte in presence of non-electrolytes, particularly certain ones having dipolar properties, large negative deviations may be produced. The concept of non-solvent bound water has no meaning in case of negative deviations such as shown here. A physical interpretation is needed which considers both positive and negative deviations. These deviations are in accord with equations 5 and 6 and experimental work reported in the literature (**9, 40, 52, 53, 54, 55, 56, 62**).

The complex nature of the interacting forces within true solutions may be illustrated by examples from the literature as shown in table VIII. Column 1 groups the table for description; column 2 gives the composition of the solvent; column 3, the kind of solute added; column 4, the deviation as determined by the method listed in column 5. Group (A) illustrates the interaction of ions and non-electrolytes. The interaction of ions and the undissociated parts of weak electrolytes is shown in group (B). The remaining examples portray the interaction between ions and dipolar compounds in which concentration of ions and structure of dipolar substances are shown to play a part. In this category, group (C) represents the behavior of a dipolar substance in the presence of monovalent ions. The effect

of concentration of KCl is exhibited in (D). Group (E) illustrates the effect of structure when a six-carbon acid with the same dipole moment as that in (C) and (D) is introduced into a solution of monovalent ions. Valence and structure effects may be noted by appropriate comparisons in (E), (F), (G), and (H). The influence of ion concentration is shown in (J) and (K) while both ion concentration and structure of dipolar compound may be seen in the last. *These are all non-colloidal solutions.*

It appears that the problem of deviations observed here is characteristic of solutions in general and that the conclusion of HÜCKEL (**30**) regarding electrolyte solutions may be extended to dilute colloidal solutions. This leads to the conclusion: *hydration is by no means as important in determining the characteristics of a solution as we are prone to think; the significance of the observed phenomena lies in the electrical forces which permeate the solution.*

The preceding discussion has dealt with phenomena in dilute colloidal solutions, more or less complex in composition, and has emphasized the relative influence of components other than the colloidal substances. An important part of the literature on bound water has dealt with systems at low water content. ROSENBOHM (**58**) found that of the thirty-six calories evolved per gram of gelatin in process of saturation, thirty-three calories were given off in the absorption of 0.24 grams of water. KATZ (**36, 37**) showed that a typical sigmoid curve, as represented in figure 1, described the relationship between relative vapor pressure and water content for

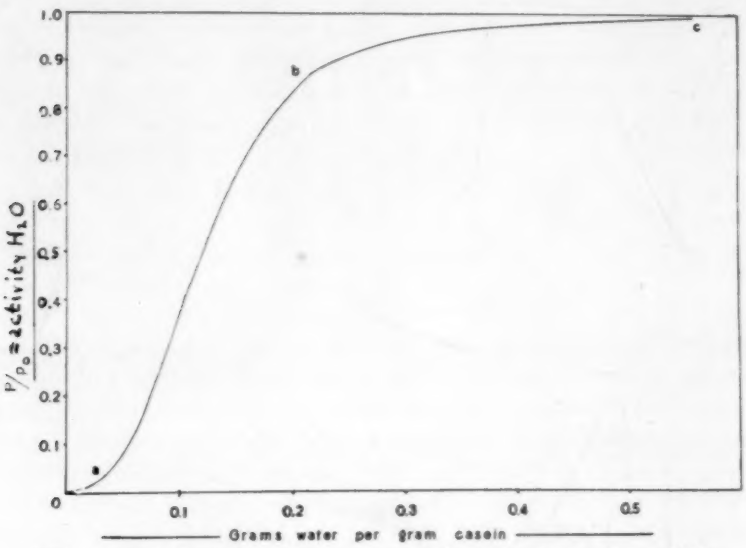

FIG. 1. Typical relative vapor pressure-water content curve for colloids.

many elastic gels. FRICKE and LÜKE (**15**) made a vapor pressure study on casein and agar at low water content. Values for partial molal free energy ($\overline{F}$), heat content ($\overline{H}$) and entropy ($\overline{S}$) calculated from their data are presented in table IX. An important decrease in entropy below the point *b* on the curve is characteristic. An interesting comparison of energy relations for water in colloids at low water content and for water contained in crystal hydrates may be made from tables IX and X. Above a water content of 0.4 or 0.5 grams per gram colloid, the water in colloidal solutions appears to behave approximately as in dilute true solutions. Similar values for bound water of this type have been found by diverse methods (**1, 22, 23, 29**). The great differences between heat content and entropy of one region and these same functions for the other region suggest that the water molecules are subjected to quite different forces in these two regions. It is justifiable to differentiate two classes of phenomena: first, those that occur in solutions wherein the process approaches reversibility; secondly, those that occur in colloidal solutions at low water content where the process is irreversible. In the first of these it appears that the forces

TABLE IX

ENERGY RELATIONS IN COLLOID SYSTEMS

GRAMS WATER PER GRAM COLLOID	SYSTEM	$\overline{F}$	$\overline{H}$	$\overline{S}$	REFERENCE
gm.					
0.059	H_2O-agar	−2214.0	−3436.0	−4.48	(15)
0.125	"	−1177.0	−2502.0	−4.85	"
0.157	"	− 957.0	−2023.0	−3.90	"
0.366	"	− 160.0	− 540.0	−1.39	"
0.478	"	− 60.0	− 75.0	−0.05	"
0.049	H_2O-casein	−1267.0	−2297.0	−3.77	"
0.143	"	− 369.0	−1063.0	−2.54	"
0.322	"	− 31.0	− 110.0	−0.29	"
0.437	"	− 8.0	− 28.0	0.13	"
27.96	H_2O-gelatin	0.3	4.6	0.04	Table IV
27.96	H_2O-gelatin KCl	0.2	11.6	0.02	Table IV

are predominantly electrical forces existing between components of the solution which tend to make it non-ideal. In the second case one deals with water in a condition similar to that of a crystal hydrate.

The evidence indicates that the chief influence upon the thermodynamic activity of water in a complex colloidal solution is the osmotic force which is not included in the definition used here for bound water. It is indicated that the electrical forces existing between the components of the solution which make it non-ideal are responsible for the phenomena often considered

as a measure of water "bound" by colloids. Under certain conditions the nature of the reference solute may profoundly affect the results obtained in measurements. In a colloidal system of low water content the thermodynamic properties of the water differ from that of the pure solvent.

TABLE X

ENERGY RELATIONS OF WATER IN CRYSTAL HYDRATES

MOL FRACTION H_2O	SYSTEM	$\overline{F}$	$\overline{H}$	$\overline{S}$	REFERENCE
mols					
0.333	$CuSO_4 \cdot H_2O$	−2004	−2783	− 2.61	(70)
0.691	$CuSO_4 \cdot 5H_2O$	− 654	−3051	− 8.04	(4)
0.706	$CdSO_4 \cdot 8/3H_2O$	− 165	−1732	− 5.26	"
0.777	$MgSO_4 \cdot 7\ H_2O$	− 365	−4036	−12.18	"

Summary

Measurements of the vapor pressure lowering and freezing point depression of some true and colloidal solutions were made and the thermodynamic behavior of these solutions has been considered.

A new interpretation of some bound water phenomena has been presented.

Results indicate that bound water in colloidal systems may be of two kinds:

(a) A small amount of water intimately associated with the colloid and having special thermodynamic properties, and

(b) A hypothetical amount of water estimated to account for the differences between the value of a property of water as found in a complex solution and the corresponding value in simple solution.

The second kind of bound water may be exhibited by any complex solution, colloidal or non-colloidal, whose components have properties which make the solution non-ideal.

The part played by colloids in this phenomenon appears to be due chiefly to their electrical properties and may be of minor importance compared with crystalloidal components.

Solvent molecules in a dilute colloidal solution show no reduction in the random orientation characteristic of the pure solvent.

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UNEQUAL DISTRIBUTION OF SOLUBLE SOLIDS IN THE PULP OF CITRUS FRUITS[1]

E. T. Bartholomew and Walton B. Sinclair

(WITH SIX FIGURES)

Introduction

The nature of the citrus fruit is such that it affords many interesting problems of research for the plant physiologist. The pulp of the citrus fruit, unlike that of most edible fruits, is divided into segments (carpels) the walls of which are not readily permeable; and each segment is composed of hundreds of units (vesicles, juice sacs) the walls of which are still more impermeable, especially laterally (**18**).

In the past, investigators have drawn many conclusions that were based entirely on results obtained by analyzing a whole plant or the major portion of a plant. While the results of such analyses have their value, they obviously omit many interesting and vital details. The results reported in this paper illustrate the value of making detailed analyses of the different tissues of a given portion of a plant. They are a part of the results of a line of research which is being carried on at the present time in this laboratory. This concerns the effects of rootstocks, maturity, insecticides, and the physiological disorder "granulation" on the composition of citrus fruit.

Materials and methods

Valencia and Navel oranges of different sizes and of different stages of maturity were used in these tests, primarily; but a few grapefruit were tested also. All mature fruits were peeled, the segments isolated, and the juice from each stem and stylar segment half, or from each entire segment, was tested for the components mentioned in later sections of this paper. When a fruit was too young to be peeled and to have the segments isolated, the entire fruit pulp was bisected transversely. The peel of such fruits was first cut away with a knife, care being taken to leave a minimum of the peel and to cut away a minimum of the pulp.

Juice was extracted from the whole or halved segments of the mature fruits with a modified, old-style lemon squeezer. From the pulp halves of the young fruits, the juice was extracted by subjecting each half to a pressure of 6,000 pounds in a hydraulic press. Aliquots of the juice were centrifuged for three minutes at 1,700 r.p.m. before being tested.

Total soluble solids were determined with an Abbé refractometer. Total acidity was determined in terms of citric acid by titrating with standardized

[1] Paper no. 423, University of California Citrus Experiment Station.

solutions of NaOH, with phenolphthalein as indicator. The pH values were determined with a Beckman glass electrode pH meter.

Sugar determinations were made by the HAGEDORN and JENSEN method (**13, 14**) as modified by BLISH (**4, 5**). The strength of the reagents employed by BLISH was satisfactory for determining the reducing and total sugars as glucose, when the values ranged from 3 to 10 mg. in 10 ml. of citrus juice. The samples were diluted when necessary, so that the values fell within this range. This method was used because comparative tests showed that it was more rapid than the best of the copper reduction methods and, at the same time gave accurate results. Because potassium ferricyanide is subject to gradual change on standing, the glucose factor was redetermined just before making each group of tests. The values were determined by checking against a sample of glucose furnished by the National Bureau of Standards.

Results

SOLUBLE SOLIDS IN CARPELLARY SEGMENTS OF CITRUS FRUITS

SOLUBLE SOLIDS IN STEM AND STYLAR HALVES OF SEGMENTS.—SUTHERST (**20**), CHACE and CHURCH (**6**), BAKER (**2**), and HAAS and KLOTZ (**12**), who made an extensive study of polarity gradients, found that the concentration of soluble solids is noticeably greater in the stylar end of certain mature citrus fruits than in the stem end. This was especially true in the Valencia orange.

As is well known, other conditions being equal, the higher the concentration of soluble solids in a liquid, the lower the temperature required to freeze it. Therefore, in cutting many thousands of mature Valencia oranges during the study of granulation [a physiological disorder of Valencia fruits (**3**)], it was surprising to find that during the previous winter one or more segments in some of the fruits had been frozen at the stylar end and not at the stem end.

In order to find an answer to this apparent anomaly, an effort was made to correlate this condition with the distribution of soluble solids in the juice of the stem and stylar ends of the fruit during the winter months before the fruit was mature. Valencia and Navel oranges and also a few grapefruits were tested. This study entailed the making of almost 3,000 refractometric readings in addition to a large number of acid, sugar, and other determinations. Most of the fruits were tested on the day they were picked or on the following day.

In the localities from which the different fruits were obtained, the Valencias mature from the last of March to the first of May; Navels, from the middle of November to the latter part of December; and grapefruit, from the last of April to the first of June. All of these citrus fruits set at approximately the same time; therefore Valencias and grapefruit mature in

12 to 14 months and Navels in 8 to 9 months. The dates of maturity have an important bearing on the interpretation of the data to be presented.

The results of determining the total soluble solids in separate segment halves of Valencias, Navels, and grapefruits are presented in table I. These

TABLE I

DISTRIBUTION OF SOLUBLE SOLIDS IN STEM AND STYLAR HALVES OF CARPELLARY SEGMENTS OF CITRUS FRUITS IN DIFFERENT STAGES OF MATURITY

DATE FRUIT WAS PICKED AND TESTED	APPROXIMATE AGE OF FRUIT	NUMBER OF FRUITS	NUMBER OF SEGMENTS	PERCENTAGE OF SEGMENTS		
				TOTAL SOLUBLE SOLIDS HIGHER IN STEM THAN IN STYLAR HALVES	TOTAL SOLUBLE SOLIDS HIGHER IN STYLAR THAN IN STEM HALVES	TOTAL SOLUBLE SOLIDS EQUAL IN STEM AND STYLAR HALVES
Valencia oranges						
	mo.			%	%	%
1937						
Nov. 1, 2	6.0	8	81	47	44	9
Dec. 27, 28	8.0	12	131	22	63	15
1938						
Jan. 5, 7, 8	8.0	9	95	25	63	12
Jan. 14–17	8.5	5	51	20	68	12
Feb. 4, 16	9.0	12	121	7	87	6
Mar. 11, 18	10.5	12	120	0	99	1
Mar. 21	11.0	12	129	1	99	0
June 28	14.0	10	106	0	100	0
1939						
Jan. 18	8.5	14	138	4	95	1
Navel oranges						
1937						
Oct. 25–28	6.0	12	130	31	53	16
Dec. 22	8.0	6	63	0	97	3
1938						
Jan. 10, 11, 31	8.5	8	76	1	99	0
Feb. 2, 9, 23, 24	9.5	7	69	0	99	1
Grapefruit						
1938						
Jan. 12–14	8.5	6	71	9	78	13
Feb. 14, 28	9.5	3	39	0	97	3

data show that, as a rule, the more mature the fruit, the greater the percentage of segments in which the total soluble solids were higher in the stylar than in the stem halves. For example, only 44 per cent. of the segments of the Valencias picked November 1 and 2, 1937, (6-month-old fruits), showed a concentration of total soluble solids higher in stylar than in stem halves, whereas practically 100 per cent. of the segments of the fruits picked from

March 11 to June 28, 1938 (10- to 14-month-old fruits), showed this condition. Similar trends were exhibited by the Navels and grapefruits.

The ages of the fruits given in table I are only approximate. The setting and maturing dates may be as much as a month or six weeks earlier or later one year than another. For instance, the Valencias tested January 14–17, 1938, were less mature than those tested January 18, 1939. This was shown, not only by the fact that on the latter date a larger percentage of the stylar halves of the segments contained the higher concentration of soluble solids (table I), but by the fact that these fruits had a considerably higher concentration of soluble solids (data not included in table) than those tested January 14–17, 1938.

From the data (table I) one may logically conclude that the concentration of total soluble solids in the stylar half of the citrus fruit does not noticeably exceed that in the stem half until the fruit is nearing maturity. It should be pointed out, however, that even in the youngest of the Valencias tested (6-month-old fruits), 44 per cent. of the segments had a higher concentration of total soluble solids in stylar than in stem halves, and 9 per cent. had equal concentrations in both halves. A similar condition existed in the Navel orange segments.

The diagrams in figures 1, 2, 3, and 4 illustrate the differences in concentration of total soluble solids in segments of Valencias about five months before maturity (fruit no. 103, fig. 1) and about two months after maturity (fruit no. 84, fig. 2), and in Navels about two months before maturity (fruit no. 92, fig. 3) and about two months after maturity (fruit no. 47, fig. 4). These two Valencias and two Navels showed the greatest extremes of all the fruits tested. Valencia no. 103 (fig. 1) had only one segment (no. 6) which

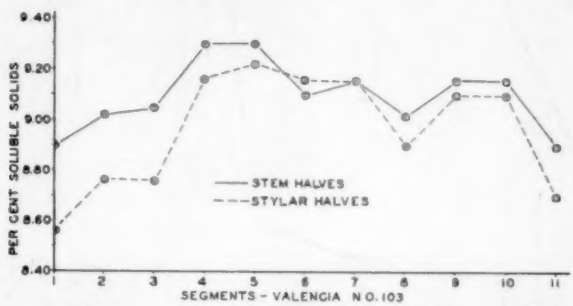

FIG. 1. Concentrations of total soluble solids in the stem and stylar halves of carpellary segments of an immature Valencia orange. With two exceptions, the concentrations are higher in the stem halves.

had a higher concentration of total soluble solids in its stylar than in its stem half. Segment 7 had the same concentration in both halves (9.16 per cent.), but all the other segments had lower concentrations in the stylar than

in the stem halves. Navel no. 92 (fig. 3) showed a condition similar to that in Valencia no. 103 (fig. 1), except that four segments (nos. 1, 2, 9, and 10)

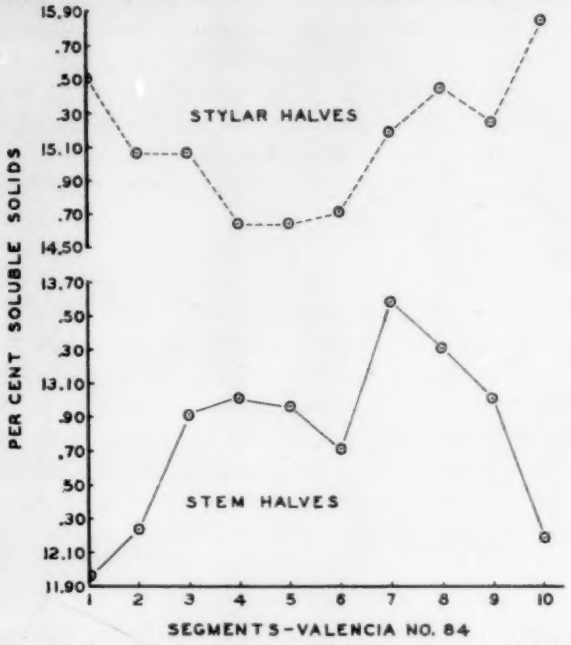

FIG. 2. Concentrations of total soluble solids in the stem and stylar halves of the carpellary segments of a mature Valencia orange. The concentrations are much lower in the stem than in the stylar halves, and there is a great difference in concentrations, not only in different segments, but in different stem and stylar segment halves.

had equal concentrations of soluble solids in both halves, and in no segment was the concentration higher in the stylar than in the stem half. The mature Valencia (fruit no. 84, fig. 2) and Navel (fruit no. 47, fig. 4) not only showed great differences in concentration in different stem and stylar segment halves, but showed much greater concentrations in all stylar halves than in stem halves. In the mature Valencia, segment 10 showed the great-

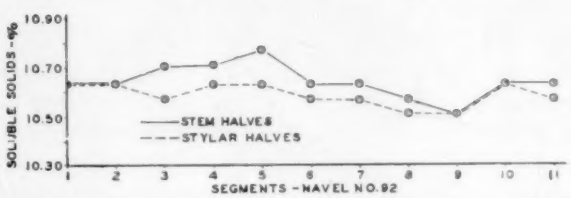

FIG. 3. Concentrations of total soluble solids in the stem and stylar halves of the carpellary segments of an immature Navel orange. The concentrations are higher in the stem halves of all segments except 1, 2, 9, and 10, in which they are equal in the two halves.

est difference between the concentration of soluble solids in the stem and stylar halves; the concentration in the stem half was 12.18 per cent. and that in the stylar half, 15.84 per cent., a difference of 3.66 per cent. A comparison of halves of segment 2 of the mature Navel showed the concentration in

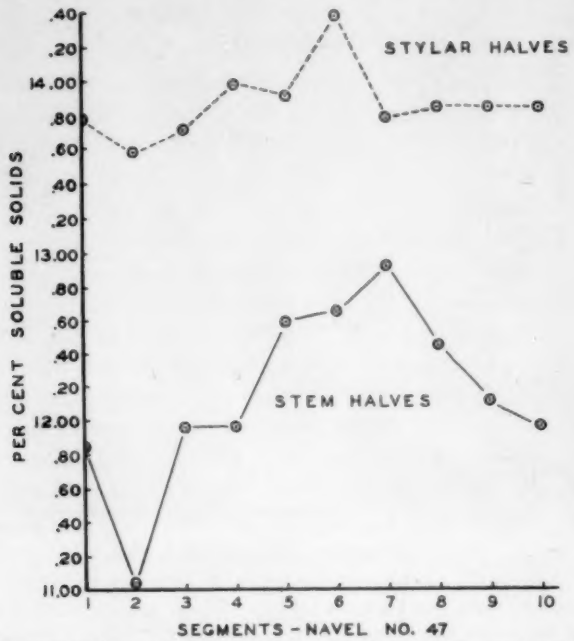

FIG. 4. Concentrations of total soluble solids in the stem and stylar halves of the carpellary segments of a mature Navel orange. As in the Valencia orange (fig. 2), the concentrations in the stylar halves are considerably higher than those in the stem halves; but the concentrations in the two halves of this orange are a little less than in those of the Valencia orange.

the stem half to be 11.04 per cent. and that in the stylar half, 13.58 per cent.; a difference of 2.54 per cent.

Of all the Valencia segments tested, the one having the greatest concentration of soluble solids in the stem half over that in the stylar half was from fruit no. 135, picked January 18, 1938. In this segment the concentration in the stem half was 8.70 and in the stylar half, 7.93 per cent; a difference of 0.77. The average difference for all Valencia segments that had the higher concentration in the stem halves was 0.22 per cent. The segment having the greatest concentration in the stylar half over that in the stem half was from fruit no. 84 (fig. 2), picked June 2, 1938. The average difference for all Valencia segments that had the higher concentration in the stylar halves was 0.76 per cent.

The concentration of soluble solids in Navel segments showed a stem-half high of 12.11 per cent. and a stylar-half low of 11.31 per cent. for all segments having the greater concentration in the stem halves, a difference of 0.80 per cent. The average difference for these segments was 0.17 per cent. The stem-half low for all segments having the greater concentration in the stylar halves was 11.04 per cent. and the stylar-half high, 13.58 per cent; a difference of 2.54. The average difference for these segments was 0.33 per cent. The results of tests of grapefruit segments were similar but are not reported here because only a comparatively few fruits were tested and these were practically all of the same age. It should be borne in mind that the differences and averages given for Valencias and Navels refer to both immature and mature fruits.

One might expect to find that in immature fruits the concentration of soluble solids would be about equal in the stem and stylar halves of the segments, even though there is a much greater concentration in the stylar halves by the time the segments mature. Table I shows that there were comparatively few segments in which the concentration of soluble solids was equal in the two halves. On the other hand, the results of a limited number of tests on whole citrus fruits indicate that in the earlier stages of development the concentration of soluble solids, though not greatly, may be measurably higher in the stem half than in the stylar half. Of 13 Valencias about three and one-half months old, 9 had an average of 0.59 per cent. higher concentration of soluble solids in the stem halves than in the stylar halves of the pulp, 1 had equal concentrations in the two halves, and 3 had an average of 0.31 per cent. higher concentration in the stylar halves than in the stem halves. Of 23 Navels of similar age, 21 had an average of 0.23 per cent. higher concentration in the stem halves than in the stylar halves, and 2 had equal concentrations in the two halves. None of the 23 Navels had a higher concentration in the stylar half than in the stem half of the fruit.

The original records show that of those fruits in which the concentration of soluble solids was higher in the stem halves than in the stylar halves of the whole fruits, some had (*a*) the higher concentration of both acids and sugars in the stem half; others had (*b*) acids higher in the stem half and sugars higher in the stylar half; or (*c*) acids higher in the stem half and sugars equal in both halves; or (*d*) acids equal in both halves, but sugars higher in the stylar half; or (*e*) acids higher in the stylar half, but sugars equal in both halves. The last condition mentioned, (*e*), indicates that soluble solids other than acids and sugars are partially responsible for the conditions mentioned (*a*) to (*d*), especially in immature fruits.

When the comparative concentrations of total soluble solids in the stem and stylar segment halves were determined (table I), the total acids (in terms of citric acid) and total sugars (in terms of glucose) were also deter-

mined in the two halves of some of the segments. Because of the great amount of work that would have been involved, not all segment halves were tested for acids and sugars. The tests were made on the stem and stylar halves of 37 segments from both immature Valencias and immature Navels and on stem and stylar halves of 20 segments from mature Navels.

The approximate ages of the fruits from which these segments were taken and the results of the tests are given in table II. The data on total soluble solids are from the stem and stylar halves of whole fruits, while the data on acids and sugars are from segment halves.

TABLE II

AVERAGE PERCENTAGE OF TOTAL SOLUBLE SOLIDS IN FRUIT HALVES AND DISTRIBUTION OF ACIDS AND SUGARS IN CARPELLARY SEGMENTS OF IMMATURE VALENCIA ORANGES AND OF IMMATURE AND MATURE NAVEL ORANGES

FRUITS TESTED	AVERAGE TOTAL SOLUBLE SOLIDS IN FRUIT HALVES		PERCENTAGE OF SEGMENTS					
			ACIDS (IN TERMS OF CITRIC ACID)			SUGARS (IN TERMS OF GLUCOSE)		
	STEM HALVES	STYLAR HALVES	HIGHER IN STEM THAN IN STYLAR HALVES	HIGHER IN STYLAR THAN IN STEM HALVES	EQUAL IN STEM AND STYLAR HALVES	HIGHER IN STEM THAN IN STYLAR HALVES	HIGHER IN STYLAR THAN IN STEM HALVES	EQUAL IN STEM AND STYLAR HALVES
	%	%	%	%	%	%	%	%
Immature Valencias (8–9.5 months old)	10.22	10.54	91	9	0	18	81	1
Immature Navels (6 months old)	9.86	9.90	60	30	10	26	58	16
Mature Navels (9.5 months old)	12.29	13.30	9	91	0	0	100	0

Although the first two lots of fruit mentioned in table II were immature, the average total soluble solids was slightly higher in their stylar halves than in their stem halves. It may be stated, however, that even in these fruits, 27 per cent. of the segments had a slightly higher concentration of soluble solids in the stem than in the stylar halves. The higher concentration in the stylar halves of the last lot of Navels mentioned in the table was to be expected because the fruits were mature.

The data in table II show that although the acid content was higher in 91 and 60 per cent., respectively, of the stem segment halves of the immature Valencias and immature Navels, total sugars were higher in 81 and 58 per cent., respectively, of the stylar segment halves of the same fruits. Of the mature Navels, 91 per cent. had the higher acid content and 100 per cent. had

the higher sugar content in the stylar segment halves. These and other data in the table show that as a result of shifting or of some other process, there is a pronounced difference in the distribution of soluble solids in immature and in mature citrus fruits.

SOLUBLE SOLIDS IN ENTIRE SEGMENTS.—The differential response to low temperatures was not confined to the stem or stylar halves of the different segments. Many fruits were found in which an entire single segment had been frozen and had collapsed, whereas the other segments were unaffected. The apparent explanation for this condition was found by refractometric determinations of the total soluble solids in individual segments of many Valencia and Navel fruits.

In making approximately 1,500 tests, it was found that comparatively great differences may exist between the concentrations in the segments of a given fruit. For example, in a mature Valencia fruit (no. 75) picked June 28, 1938, one segment contained 16.11 per cent. soluble solids, while the two adjacent segments contained 13.41 and 15.44 per cent., respectively. Another segment in a different portion of the same fruit contained only 12.61 per cent.—a difference of 3.50 per cent. between the highest and lowest concentrations in the segments of that fruit.

In the stem half of one of the segments in this fruit (no. 75) the concentration of soluble solids was only 11.38 per cent., while in the stylar half of one of the other segments, the concentration was 16.98 per cent., a difference of 5.60. The differences mentioned for this fruit were greater than for any other fruit tested, but almost all the other fruits were less mature than this one.

SOLUBLE SOLIDS IN NORTH AND SOUTH SEGMENTS OF FRUITS.—Each fruit, before it was picked, was marked to designate its north or south side as it hung on the tree. It was also marked to indicate from which side of the tree it was taken and whether it was an inside or an outside fruit.

When the tabular data on concentration of total soluble solids in different segments was examined an interesting, but as yet unexplained, phenomenon was discovered. Of fruits picked from the outside of the trees and about 4 to 6 feet from the ground, 80 out of 88 Valencias, 25 out of 33 Navels, and 8 out of 9 grapefruits (or 87 per cent. of the 130 fruits) had a higher total soluble-solids content in their three north segments than in their three south segments. This condition held whether the fruits were borne on the north, south, east, or west sides of the trees, provided they were outside fruits.

Figure 5 shows the comparative concentrations of total soluble solids in each of the segments of 3 of the 80 Valencia oranges that showed a higher concentration in their three north than in their three south segments. The vertically hatched columns represent the three north, and the stippled

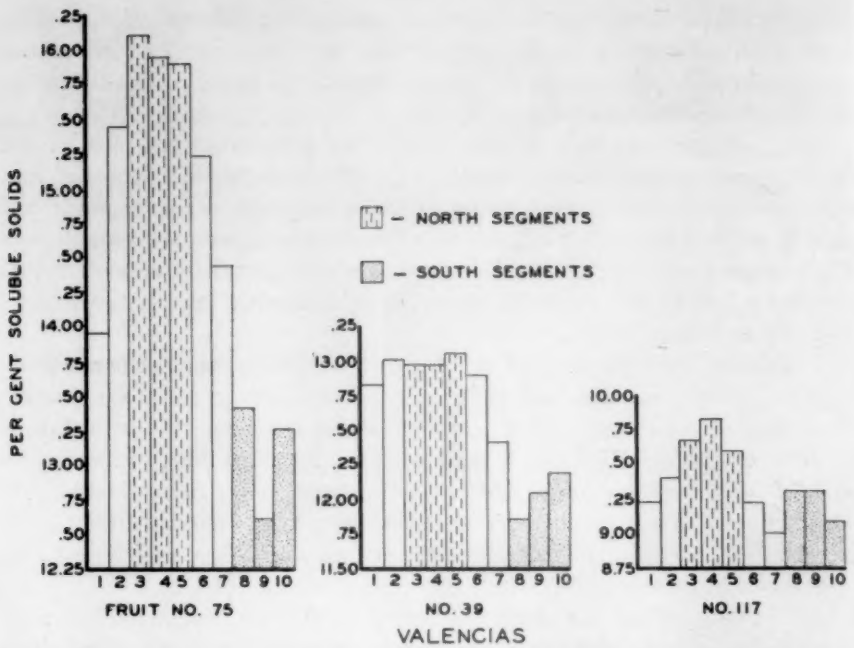

FIG. 5. Comparative concentrations of total soluble solids in the three north and the three south carpellary segments of three Valencia oranges. This figure shows the great difference in concentrations in the different segments of a given fruit.

columns, the three south segments in each fruit. The average total soluble solids for the three north segments of Valencia no. 75 was 15.98 per cent. and for the three south segments, 13.09 per cent. Averages for the three north and the three south segments of fruits nos. 39 and 117 were 12.98 and 12.02 per cent. and 9.69 and 9.23 per cent., respectively. Valencia no. 75 was picked June 28; no. 39, February 12; and no. 117, December 28. The indications are that the more mature the fruit, the greater is the difference in concentration of soluble solids in the segments, and, also, the greater is the north-south polarity of concentrations in the fruit.

Figure 6 represents the comparative total soluble solids in each of the segments of 3 of the 25 Navel oranges that showed a higher concentration in their three north than in their three south segments. The differences, however, were not so great in the Navels as in the Valencias. Navel no. 89 (fig. 6) was more mature than Valencia no. 117 (fig. 5), and Navel no. 46 (fig. 6) was less mature than Valencia no. 75 (fig. 5). Navels nos. 46, 33, and 89 were picked February 24, February 9, and October 27, respectively. The average total soluble solids for the three north segments of Navel no. 46 was 14.39 per cent. and for the three south segments, 12.91 per cent. Aver-

ages for the three north and the three south segments of Navels nos. 33 and 89 were 12.20 and 11.71 per cent. and 11.00 and 10.66 per cent., respectively.

While in 80 out of 88 Valencias and in 25 out of 33 Navels the three north segments contained greater concentrations of soluble solids than the three south segments, attention should be called to the fact that the segment which contained the highest concentration was not always on the north (fruit no.

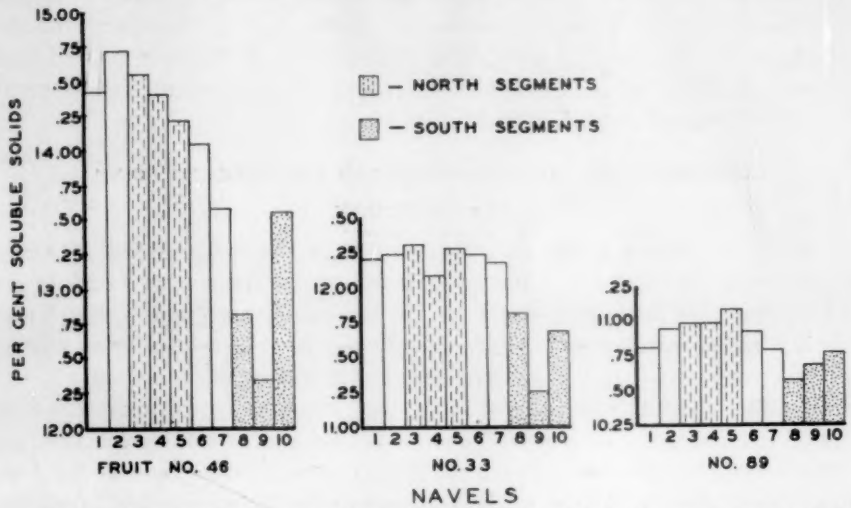

FIG. 6. Comparative concentrations of total soluble solids in the three north and the three south segments of three Navel oranges. The segment differences are similar to those in the Valencias (fig. 5), but are a little less.

46, segment 2, fig. 6), and the one that contained the lowest concentration was not always on the south side of the fruit (fruit no. 117, segment 7, fig. 5). As a rule, however, the concentration of soluble solids in the two segments adjacent to the group of three north segments was higher than that in the two segments adjacent to the group of three south segments. Figures 5 and 6 illustrate this condition.

The north-south polarity concentrations were determined in only six inside fruits, all Valencias, time not permitting the making of more such tests. Two of the six fruits showed higher concentrations of soluble solids in the three north segments than in other segments, and four showed higher concentrations in the three south segments. The exposures of inside fruits are variable; for some fruits may be heavily shaded, while others may receive almost as much light as those on the outside.

Just what significance lies in the fact that, as a rule, the north half of an outside citrus fruit contains a higher concentration of soluble solids than its south half, can be determined only by further study. The fact is interesting

from a physiological viewpoint, but with the data at hand, the condition is as difficult to explain as it is interesting.

SIZE OF SEGMENTS AND CONCENTRATION OF SOLUBLE SOLIDS.—Even by a casual observation it can be determined that the segments in the pulp of a citrus fruit are not all equal in size. Some segments are comparatively large; others may extend the entire length of the pulp, but be only about a fourth to a half as thick as the largest; and in occasional fruits, especially in navels and grapefruits, a segment may extend only a half or two-thirds of the length of the fruit and be very thin. The results of the tests showed that there is no direct relation between the size of the segment and the concentration of total soluble solids which it contains.

SOLUBLE-SOLIDS CONCENTRATION AND THE FREEZING POINT OF CITRUS JUICE

Since the concentration of soluble solids in the stylar halves of many segments of immature and newly matured citrus fruits was equal to, or lower, than that in the stem halves of the same segments (table I, figs. 1 and 3), it is not surprising that freeze injury was found in the stylar as well as in the stem halves. Nor is it surprising that freeze injury was found in one segment of a given fruit and not in another when, as shown in figures 2, 4, 5, and 6, there was such a difference in concentration of soluble solids in the different entire segments. These conditions prevail in the fruit at the time of the year when it is most likely to be subjected to freezing temperatures. Later, as already stated and as found by other investigators (**2, 6, 12, 20, 22**), the concentration of soluble solids is noticeably higher in the stylar half than in the stem half of the fruit; and it seems logical to conclude that if the fruit were subjected to freezing temperatures at this time, the injury would probably register first in the stem half.

The concentration of the total soluble solids in expressed sap does not, alone, determine the exact temperature at which this sap would have frozen while present in the tissues. Articles by other investigators in recent years have presented much data on this point, but they will not be reviewed here. Time and equipment were not available when these tests were made, to determine the freezing point of the juice *in situ* in the segments or segment halves.

A few tests were made, however, to determine whether the concentration of soluble solids in the juice expressed from immature and mature citrus-pulp tissues would give a reliable clue to the freezing-point trends of the expressed juice. The results of these tests are shown in table III. The fruits were not frozen before the juice was extracted. The peels were cut away from the immature Navels, as explained previously; the pulps were bisected transversely, and each half was subjected to 6,000 pounds pressure. The mature Valencias were bisected transversely without peeling, and the

TABLE III

SOLUBLE SOLIDS IN JUICE AND FREEZING-POINT DEPRESSION OF JUICE FROM STEM AND STYLAR HALVES OF IMMATURE NAVEL AND MATURE VALENCIA ORANGES

FRUIT NUMBER	SOLUBLE SOLIDS IN JUICE		FREEZING-POINT DEPRESSION OF JUICE	
	STEM HALF OF FRUIT	STYLAR HALF OF FRUIT	STEM HALF OF FRUIT	STYLAR HALF OF FRUIT
Navel oranges (immature)				
	%	%	°C.	°C.
1	9.07	8.95	0.989	0.980
2	9.01	8.80	0.979	0.963
3	9.15	8.95	1.001	0.997
4	9.61	9.15	1.026	1.006
5	9.61	9.35	1.048	1.019
6	9.77	9.77	1.079	1.034
7	9.35	9.35	0.966	0.962
8	9.50	9.43	0.974	0.994
9	9.69	9.49	0.981	0.967
10	9.50	9.33	0.976	0.859
11	9.89	9.43	0.968	0.960
12	9.07	8.80	0.978	0.955
Valencia oranges (mature)				
13	16.44	19.66	1.935	2.512
14	15.06	17.46	1.863	2.200
15	14.06	16.27	1.590	1.828
16	16.29	17.58	2.055	2.379
17	15.24	17.44	1.780	2.084
18	16.25	17.51	1.902	2.118
19	14.90	16.70	1.719	2.010
20	15.11	17.69	1.809	2.287

juice was reamed from each half. Soluble-solids and freezing-point determinations were made on the juice thus obtained from the two lots. The Navels used in these tests were about 4 months old, and the Valencias, about 17.5 months old. Valencias were used because no mature Navels were available at the time these tests were made.

The data in table III show that the concentration of soluble solids was slightly higher in the stem than in the stylar halves of all the immature fruits except nos. 6 and 7, in which soluble solids were equal in the two halves. The freezing points were also slightly higher for the juice from the stem halves of all the fruits except no. 8.

The extreme differences in concentrations of soluble solids and freezing-point depressions for the juice from the immature Navels were small, 1.09 per cent., and 0.175° C., respectively; but if the differences between the stem and stylar halves are considered, they appear to be significant, since the results by the two methods show the same trends. On the other hand, when the relationship between the soluble solids and the freezing-point depres-

sions is considered without reference to the differences in concentrations in the stem and stylar halves of the fruit, some discrepancies are evident. For example, the juice from the stem halves of fruits 4 and 5 had the same percentage of soluble solids but different freezing-point depressions.

The results of the tests on the mature Valencias (table III) show not only a marked increase in concentration of soluble solids over that of the immature Navels, but also concentrations much greater in the stylar than in the stem halves of the fruits. As would be expected, these increased concentrations resulted in much greater depressions of the freezing points. Here again small discrepancies may be found, however, and a straight line would not be obtained if the percentages of soluble solids were plotted against the freezing-point depressions.

The apparent discrepancies which appear in the results of these tests (table III) may have been caused by experimental error and by soluble inorganic constituents which had a marked effect on the freezing-point depression and but little effect on the refractive index of the solution. Or they may have been caused by the presence of colloidal material which registered in the refractive index but had little or no effect on the freezing-point depression.

As was previously stated, in earlier tests fruits were found in which freeze injury was evident in the stylar and not in the stem halves of the segments. These findings, together with those given in table III, indicate that if immature Navels are subjected to the minimum temperature which will cause injury, the stylar halves of the segments will be injured first.

Color differences in centrifuged juice

The supernatant portion of the centrifuged juice from the two halves of a segment did not always have the same depth of color. Data of this kind were kept on the segments of only 80 of the fruits tested, and most of these segments were from mature or nearly mature fruits. Eighteen of the fruits showed no difference in the color of the juice from the stem and from the stylar halves of the segments. Of the 654 segments of the other 62 fruits, 33 (5 per cent.) had darker juice in the stylar halves than in the stem halves; 328 (50 per cent.) had the darker juice in the stem halves; there was no appreciable difference, however, in the color of the juice from the halves of the remaining 293 segments, There were 5 fruits in which the juice from the stem half of every segment was darker than that from the stylar half.

Differences in juice color were more prevalent and more noticeable in mature than in immature fruits and were found in all three varieties of citrus fruits tested—Valencia, Navel, and grapefruit. In addition to color there were often similar differences in the juice from the whole segments of a fruit.

The degree of any color contrast was not accurately determined by the use of photoelectric or similar equipment, but was determined simply with the unaided eye. Where there was a color difference in the juice from different portions of mature fruits, it appeared that the amount of carotene present might be the controlling factor. In immature fruits the juice was pale gray with a greenish tinge. Contrasts in juice of this kind appeared to be owing to differences in opacity rather than in color.

As yet, no attempt has been made to isolate the substance or substances that are responsible for the color differences, nor is any special significance attributed at this time to their existence. Their significance, if any, and their explanation await further and more accurate tests. These data are included merely to give added evidence of the desirability and value of studying small portions of a given plant.

Discussion

The refractometer was used for determining the dry weights of the citrus juices, expressed in terms of total soluble solids, because preliminary tests showed that the results obtained by this method were as accurate as those obtained by the dry-weight method and were much more rapidly determined. To test the dry-weight method, 25-ml. aliquots of juice were dried in vacuum at 72 to 73 cm. and at a temperature of 55° C. The results of the preliminary tests are given in table IV. In three of the five tests, the results obtained

TABLE IV

COMPARISON OF PERCENTAGES OF SOLUBLE SOLIDS IN CITRUS JUICE, DETERMINED BY DRYING IN VACUUM AND WITH REFRACTOMETER

EXPERIMENT NUMBER	SOLUBLE SOLIDS IN JUICE	
	DETERMINED BY DRYING IN VACUUM*	DETERMINED WITH REFRACTOMETER
1	11.70	11.83
2	11.76	11.91
3	11.58	11.57
4	9.34	9.33
5	10.10	10.26

* Twenty-five-ml. aliquots of juice dried in vacuum at 72 to 73 cm. and at temperature of 55° C.

with the refractometer are slightly higher than those obtained by the dry-weight method. It is not likely that the differences are significant, but if they are, it is probable that the results obtained with the refractometer are the more accurate because of the time element and the effect of the vacuum on the results given by the dry-weight method. The results compare very satisfactorily with those of GORTNER and HOFFMAN (10), who used the refractometer for the determination of moisture as well as for the determina-

tion of colloidal material in expressed plant saps. Therefore, the determination of the soluble solids by the refractive index of citrus juice is considered to be a rapid and an accurate method for determining its dry weight.

An unequal distribution of soluble solids has been found in fruits other than citrus. LALL (17) and ARCHBOLD and BARTER (1) have shown that the most highly colored half of an apple, when cut from stem to calyx, and the calyx half of the transversely cut apple, may contain a higher concentration of soluble solids than the opposite halves. HARDING (15) found the concentration of soluble solids was highest in the skin of the Jonathan apple, with a gradual decrease toward the pith. TUCKER (21) and SCOTT (19) found an unequal distribution of soluble solids in different varieties of melons.

It is possible that migration or redistribution of soluble solids in such fruits as apples and melons could be more easily explained than similar processes in citrus fruits. The segment (carpel) walls in citrus fruits, especially the walls of the vesicles which contain the soluble solids, have a high degree of impermeability. REED (18) used different dyes on fresh vesicles and on those that had been treated with such substances as alcohol, ether, NaOH, and HCl and concluded that the lateral walls of the vesicles contained suberin or cutin, which made them comparatively impermeable. He found that the walls of the stalk and of the distal tip of the vesicle were more permeable than those of the body of the vesicle. From these results it would appear that to migrate from the stem end to the stylar end of the fruit, soluble solids would have to pass out of each vesicle stalk, in which there is no developed vascular tissue, through the segment wall, and into the surrounding parenchymatous tissue of the peel. From there they would have to migrate slowly through this tissue toward the stylar end of the fruit or would have to find their way to one of the bundles of the peel, where they would be transferred more rapidly. All vascular bundles of the citrus fruit are separated from the walls of the segments by at least several layers of parenchymatous tissue, except where they pass through the inner walls and into the ovules.

That the segment and vesicle walls of citrus fruits are comparatively impermeable is further indicated by the work of CURTIS and CLARK (8), who found that when the two opposite sides of apples and tomatoes were kept at different temperatures, there was a movement of water, in vapor form, from the warm to the cool side. No such movement of water could be induced in oranges and potatoes by the same treatment. For a more extended discussion of the work on apples, see CURTIS (7). It may be mentioned here that similar tests had been made on oranges at the Citrus Experiment Station before it was known that CURTIS and CLARK had done their work. The results were the same as those obtained by these men. The nature of the tissues in

the pulp of the orange is such that the movement of water is probably prevented largely by the comparative impermeability of the walls rather than merely by the absence of air spaces, as CURTIS and CLARK concluded.

Several other theories might be advanced to explain the presence of a higher concentration of soluble solids in the stylar than in the stem end of mature citrus fruits. For example, there may be an uneven rate of respiratory activity in the two ends of the fruit as it matures, or the soluble solids may naturally continue to flow in greater abundance to the tissues that have been most recently in a meristematic condition. The navel portion of the Navel orange contains a higher concentration of soluble solids than any other portion of the mature fruit, and it is the youngest portion of the Navel fruit.

Inasmuch as there is no transfer of substance from one segment to another and each segment behaves principally as a separate unit, it is not surprising that one segment may have a higher concentration of soluble solids than another, even the adjoining one, in the same fruit. This condition fits in very well with the theory that each carpellary segment of the pulp of a citrus fruit represents a modified leaf, because no two leaves on a twig contain the same concentration of soluble solids. It is not the purpose of this paper, however, to present any argument for or against any theory concerning the ontogeny of citrus or other fruits. For recent discussions of three different theories on this subject, see EAMES and MACDANIELS, (**9**); HAYWARD, (**16**); and GREGOIRE, (**11**).

Sufficient data are not yet available to explain the north-south polarity of concentration of soluble solids in citrus fruits. That the different amounts of light received by the two sides of the fruit may be a factor is indicated by the fact that the difference in concentrations in the north and south halves of the fruit is usually greater in the fruits from the south than in those from the north side of the tree. Just why the shaded side of the fruit on the south side of the tree and the exposed side of the fruit on the north side of the tree should have the highest concentration is difficult to explain, unless one concludes that the south side of the south, east, and west fruits receive more direct light and, therefore, more heat, and that the resulting increase in respiration causes a decrease in total concentration of soluble solids in the south half over that in the north half of the fruit. This could not be true for the north fruit however, unless one were to conclude that the south half, though shaded, was warmer than the north half.

A study of the vascular system of the citrus fruit does not afford any direct explanation for the condition under discussion. A transection through the center of the fruit shows as many vascular bundles in the center of the fruit as there are segments (carpels). Each bundle, as a rule, lies near the inner point of contact of each two adjacent segment walls. Branches from these bundles pass into surrounding parenchymatous tissue

and into the ovules, but they do not pass through or even contact the segment walls except at the point of attachment of the ovules. One large bundle may be seen in the mesocarp (spongy parenchyma of the peel) in the vicinity of the center of the external wall of each segment; another, in a similar position but in the depression opposite the juncture of two segment walls. Branches from these main bundles pass into all parts of the mesocarp of the peel, but they do not penetrate or contact the tissues of the segment walls. The lack of direct contact of any of the vascular bundles with any of the vesicles, or even with the segment wall which surrounds the vesicles, would at least lead one to question the theory that the differences in concentration of soluble solids in different segments or parts of segments, are due to the difference in amounts of food or food materials supplied by the different vascular bundles of the fruit. It is probable that an explanation would more likely be found by a study of conditions inherent in the segments themselves.

Summary

Mature oranges and grapefruit have a considerably higher concentration of total soluble solids in their stylar than in their stem halves. Immature fruits may have equal amounts in both halves or may have the higher concentration in their stem halves. The latter condition suggests an explanation for the fact that the stylar half of an immature fruit segment may be injured by low temperature while the stem half may remain uninjured.

The concentration of total soluble solids in the segments of a given fruit may differ greatly, especially if the fruit is mature. This may be largely because of the comparatively impermeable nature of the walls of the segments and juice vesicles.

Of 130 fruits tested, 87 per cent. had a higher concentration of total soluble solids in their three north than in their three south segments. This condition was found whether the fruits were borne on the north, south, east, or west side of the tree, provided they were exposed, outside fruits.

Apparently the concentration of soluble solids in a segment of a citrus fruit is not governed by the size of the segment.

The total soluble-solids content of expressed citrus juice, determined with the refractometer, appears to be a reliable index to the temperature at which such juice will freeze.

In the citrus fruits tested, the color of the juice was not the same in all the segments of a given fruit, nor was the color always the same in the stem and stylar halves of the segments.

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TOLERANCE OF SHORTLEAF PINE SEEDLINGS FOR SOME VARIATIONS IN SOLUBLE CALCIUM AND H-ION CONCENTRATION[1]

A. G. CHAPMAN

(WITH FIVE FIGURES)

Introduction

Shortleaf pine, *Pinus echinata* (Mill.), is known to be limited in its natural distribution to low-calcium, acid soils. The species occurs principally on residual sandstone and shale soils and on acid, cherty soils, low in soluble basic compounds, derived by weathering from highly siliceous limestones. A few areas in the Central States region have high-calcium, acid soils which have been derived from calcium sulphate, or gypsum. Such areas are small and relatively unimportant in the portion of the region within which shortleaf pine occurs. Failures of seedlings at and soon after emergence have been experienced in attempts to produce planting stock in newly established forest nurseries containing neutral or alkaline soils high in soluble calcium. In other nurseries, failures have been attributed to accumulation of basic compounds in the soil, resulting from sprinkling with alkaline water having a high calcium content. Apparently, the adverse effects of high-calcium neutral or alkaline soils on the seedlings are restricted to the early stages of seedling development; when seedlings of plantable size developed under favorable nursery soil conditions have been transplanted to neutral or alkaline field soils with relatively high calcium, they have survived and grown quite acceptably.

Apparently little attention has been given to shortleaf pine's specific ranges of tolerance for soluble calcium and hydrogen-ion concentration. ILLICK and AUGANBAUGH (**5**), in studies on pitch pine, *Pinus rigida* (Mill.), observed that "for best results, it should have acid soil in the nursery. If there is an excess of lime in the soil, seedlings turn yellow. . . . Pitch pine . . . almost never occurs naturally on limestone soils. . . . It is able to make good growth when planted thereon. . . . An excess of lime in the soil creates conditions which are most favorable for the activities of damping-off fungi. . . . Under ordinary conditions, (seedlings) would occur there naturally, were they able to survive damping-off during the first six months after germination." They did not mention any direct effects of excess of lime upon the seedlings. After investigations on ponderosa pine, *P. ponderosa* (Dougl.), HOWELL (**4**) concluded that "calcium is not dele-

[1] A contribution from the Central States Forest Experiment Station, U. S. Forest Service, Columbus, Ohio.

terious to the seedlings. The fact that the plant does not assimilate the ion in alkaline soils may be of more importance." He found indications that seedlings of the species grow best in an acid medium, but that under field conditions other factors may have more influence on growth than the soil reaction.

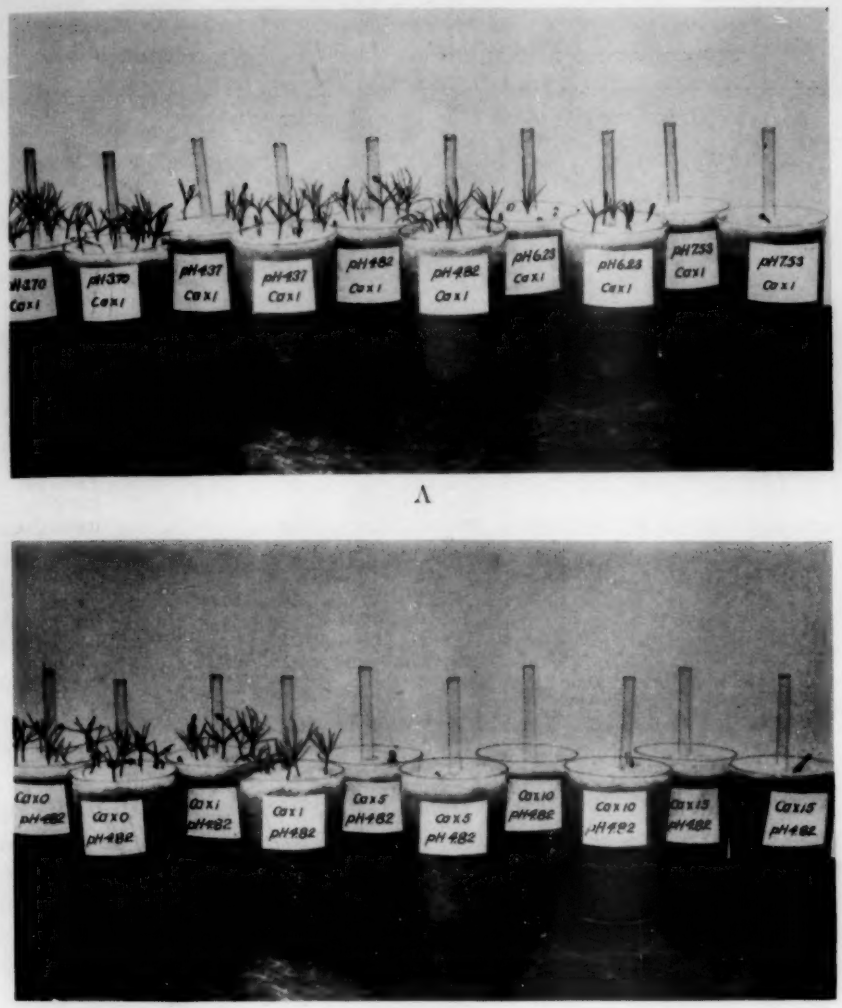
The purpose of the present investigation was to determine whatever direct effects soluble calcium and hydrogen-ion concentration may have upon young seedlings of shortleaf pine and the ranges of tolerance of the seedlings for soluble calcium and hydrogen-ion concentration. The experiments were conducted in the greenhouse of the Department of Botany, Ohio State University, in the spring of 1937, and in the Forest Service nursery near Chillicothe, Ohio, in the summer of that year.

Materials and methods

Two 5-unit series of cultures of shortleaf pine, each in duplicate (fig. 1), were set up in average-sized drinking glasses under greenhouse conditions and maintained for 18 days. The culture media of series A had a common level of calcium, 249 p.p.m., in solution but had pH values of 3.7, 4.4, 4.8, 6.2, and 7.5; those of series B had a common pH value of 4.8, but their calcium levels were no calcium (except possible impurities in C.P. compounds) and 249, 1,245, 2,490, and 3,735 p.p.m. The excessive amounts of calcium were employed because at the time of the experiment no indication of seedling tolerance for calcium was available.

Aside from differences in the culture solutions, the several units were similar in construction. In each glass 250 ml. of washed quartz sand had been placed, and in the center of each was a glass watering tube, held upright by the sand and extending to the bottom. The sand in each glass was watered with 80 ml. of a culture solution. Twelve shortleaf pine seeds which had begun to germinate between layers of moist cheesecloth were distributed over the sand surface and covered with 30 ml. of dry sand. The quantity of culture solution was sufficient to bring the "solution table" to a point $1\frac{1}{4}$ inches below the surface and to maintain the surface layer in a moist—but not waterlogged—condition.

The stock solutions used were modifications of the buffered solutions used by HOPKINS and WANN (3) for culturing *Chlorella* sp. Their composition is indicated in table I. The amount of calcium used in any stock solution is indicated in terms of the quantity contained in the "balanced" culture solution, solution A. Solution A and the phosphate buffer solutions are identical with those used by HOPKINS and WANN. Solution B contains no calcium, and contains nitrogen in the form of ammonium nitrate. Solutions C, D, and E have nitrogen added in the form of ammonium nitrate and calcium in the form of calcium acetate. Table II indicates in columns



A

B

FIG. 1. Cultures of shortleaf pine seedlings, each in duplicate: A, in uniform water-soluble calcium and varied H-ion concentration; and B, in uniform H-ion concentration and varied water-soluble calcium.

3 to 6 the quantities and combinations of the solutions listed in table I which were used to obtain the final culture solutions.

The original solution-table levels were uniformly maintained throughout the two series, by daily adding distilled water through the watering tube. Only the initial 80 ml. of culture solution was applied to any unit during the experiment. After 18 days, none of the pH values had changed more

TABLE I

STOCK SOLUTIONS FROM WHICH CULTURE SOLUTIONS WERE DERIVED

STOCK SOLUTION	COMPOSITION	
Solution A—(Ca × 1)	$Ca(NO_3)2 \cdot H_2O$	2.95 gm.
	$MgSO_4 \cdot 7\ H_2O$	0.40 gm.
	$Fe_2(SO_4)3$	0.005 gm.
	Distilled water to	1,000 ml.
Solution B—(Ca × 0)	NH_4NO_3	2.00 gm.
	$MgSO_4 \cdot 7\ H_2O$	0.40 gm.
	$Fe_2(SO_4)3$	0.005 gm.
	Distilled water to	1,000 ml.
Solution C—(Ca × 5)	$Ca(C_2H_3O_2)2 \cdot H_2O$	10.296 gm.
	NH_4NO_3	2.000 gm.
	$MgSO_4 \cdot 7\ H_2O$	0.400 gm.
	$Fe_2(SO_4)3$	0.005 gm.
	Distilled water to	1,000 ml.
Solution D—(Ca × 10)	$Ca(C_2H_3O_2)2 \cdot H_2O$	21.580 gm.
	NH_4NO_3	2.000 gm.
	$MgSO_4 \cdot 7\ H_2O$	0.400 gm.
	$Fe_2(SO_4)3$	0.005 gm.
	Distilled water to	1,000 ml.
Solution E—(Ca × 15)	$Ca(C_2H_3O_2)2 \cdot H_2O$	32.984 gm.
	NH_4NO_3	2.000 gm.
	$MgSO_4 \cdot 7\ H_2O$	0.400 gm.
	$Fe_2(SO_4)3$	0.005 gm.
	Distilled water to	1,000 ml.
Phosphate buffer solutions		
1	0.1333 mol. H_3PO_4	13.07 gm. per liter.
2	0.1333 mol. KH_2PO_4	18.156 gm. per liter.
3	0.1333 mol. K_2HPO_4	23.23 gm. per liter.

than 0.2 unit. Seed and seedling mortalities for the several cultures during the 18 days, represented in figure 1, were used as the basis of judging the effects of the various gradations of calcium content and hydrogen-ion concentration. Differences among them were tested for significance by Chi square (χ^2).

To observe any possible plasmolysis of cells, fresh root-tip sections from seedlings a few weeks old grown in acid soil media were placed in hollow ground slides for 2 hours and then examined under a microscope (1) in a series of solutions with a pH value of 4.8, ranging in calcium content from 249 to 1,245 parts per million, (2) in the solution of series A having a pH value of 6.23, as presented in table II, and (3) in distilled water. The series of solutions, the calcium content of which ranged from 1 to 5 times the amount in the "balanced" solution, were employed in order that the point of plasmolysis, if any, might be determined rather definitely. Simultaneously, germinating seeds were placed on a series of filter papers saturated with the several solutions for observation of any toxic effects.

Osmotic values of the solutions in which tests were made for plasmolytic

TABLE II

MIXTURES OF SOLUTIONS LISTED IN TABLE I TO OBTAIN THE FIVE LEVELS OF pH AND OF CALCIUM IN CULTURE SOLUTIONS

INITIAL pH (ELECTROMETRIC)	CALCIUM	SOLUTION AND VOLUME	0.133 MOL. H_3PO_4	0.133 MOL. KH_2PO_4	0.133 MOL. K_2HPO_4
Series A	*p.p.m.*	*ml.*	*ml.*	*ml.*	*ml.*
3.70	249	A–125	10	115.00	.....
4.37	249	A–125	.....	125.00	.....
4.82	249	A–125	.....	121.88	3.13
6.23	249	A–125	.....	87.50	37.50
7.53	249	A–125	.....	25.00	100.00
Series B					
4.82	0	B–125	.....	121.88	3.13
4.82	249	A–125	.....	121.88	3.13
4.82	1245	C–125	.....	121.88	3.13
4.82	2490	D–125	.....	121.88	3.13
4.82	3735	E–125	.....	121.88	3.13

action on root cells were determined by the cryoscopic method. For comparison with these values, osmotic values of a solution of alkaline nursery soil in which germinating seed had died and of the expressed saps of roots and tops of seedlings from the same lot were obtained.

To determine possible effects of the hydrogen-ion concentrations of soil media upon those of sap of shortleaf pine seedling roots, pH values of expressed saps from seedling roots grown in soils of different acidity were determined.

Measurements of the buffer capacities of two lots of expressed root sap of seedlings a few weeks old were made on 10-ml. samples by titration against N/10 solutions of HCl and NaOH. The roots used were from two lots of seedlings grown in a single nursery bed in an alkaline soil having a pH of 7.1 and an acid soil having a pH of 5.6. Only a few yellow-green seedlings still survived in the alkaline soil. Drops of N/10 HCl were thoroughly mixed with a sap sample, one by one, until the pH was lowered to approximately 3.5, pH being recorded after the addition of each drop. Similarly, a N/10 NaOH solution was mixed with a sap sample until the pH value was increased to 8.2, pH being recorded after the addition of each drop. The results of the titrations were plotted on semilogarithmic paper, pH values on the logarithmic scale and volume of added solutions on the arithmetic scale (fig. 2).

In an effort to increase the buffer capacity of the cell sap of roots of seedlings growing in nursery soil, superphosphate was applied to plots of seedlings developing in a section of a bed where the soil had a pH value of 5.5. Other plots of seedlings in the same section of bed were not treated. After a month, roots of seedlings from the fertilized plots and from the

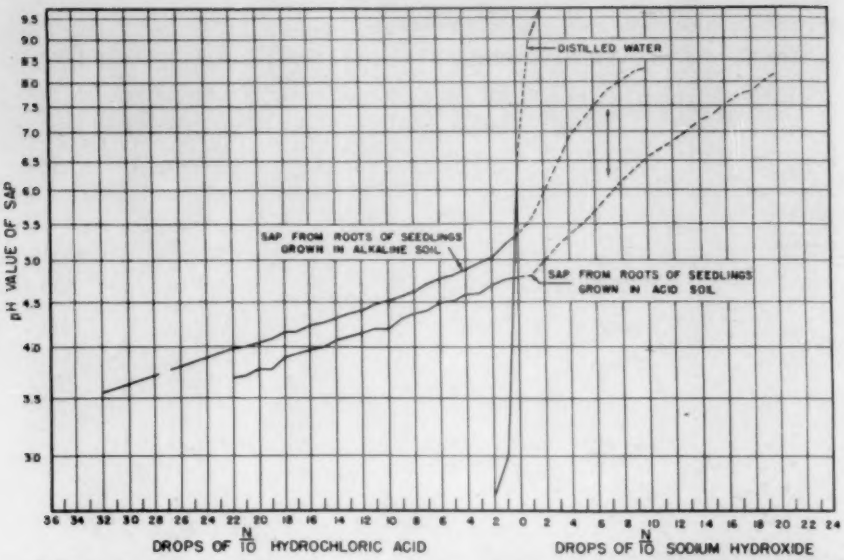

DISTILLED WATER
SAP FROM ROOTS OF SEEDLINGS GROWN IN ALKALINE SOIL
SAP FROM ROOTS OF SEEDLINGS GROWN IN ACID SOIL
pH VALUE OF SAP
DROPS OF N/10 HYDROCHLORIC ACID
DROPS OF N/10 SODIUM HYDROXIDE

FIG. 2. Results of titration of two lots of sap expressed from roots of shortleaf pine seedlings grown in acid (pH 5.6) soil and in alkaline (pH 7.1) soil against N/10 HCl and N/10 NaOH. Trends of curves indicate relative buffer capacities.

unfertilized plots were lifted and tested for buffer capacity in the manner described for tests on seedlings growing in acid and alkaline soils. Colorimetric phosphorus tests were made on samples of expressed sap from the two lots of seedling roots.

Amount of water-soluble calcium was determined on the nursery soils which had proved toxic to seedlings and on those which had not, for comparison with the amounts of soluble calcium in the culture solutions indicated in table II.

Results

GREENHOUSE SAND CULTURES

The results of the sand cultures are presented in figure 1 and in table III. Figure 1, A, represents the results of the tests on shortleaf pine seed and seedlings in culture media with five levels of pH and uniform calcium at the end of 18 days. Original pH is indicated on each container, varying from 3.70 through 4.37, 4.82, and 6.23 to 7.53. It may readily be observed that no notable effect was produced upon seedling behavior by successively higher degrees of alkalinity up to and including pH 4.82. In the pair of cultures having a pH of 6.23, seedling growth rate was low and mortality was high. In the culture medium with pH 7.53, 18 of the 24 seeds continued to germinate until emergence, but all seedlings died. When the

behavior of seedlings in cultures having pH values of 6.23 and 7.53 is compared with that of seedlings in the three cultures having lower values (table III, series A), it is clear that the differential was greater in mortality of seedlings than in mortality of germinating seed. It should not be overlooked, however, that germination was lower in the high-pH media than in the low. In regard to series A, the hypothesis was that the behavior of both seed and seedlings in all cultures did not differ from that of the control culture, with pH 4.82. The Chi-square (χ^2) tests indicated that only the seed and seedlings in cultures having pH values of 6.23 and 7.53 behaved in a way differing significantly from that of the control.

TABLE III

CHI SQUARE (χ^2) TESTS OF SIGNIFICANCE OF DIFFERENCES* IN BEHAVIOR OF SEED AND SEEDLINGS IN UNIFORM CALCIUM AND VARIED H-ION CONCENTRATION AND IN UNIFORM H-ION CONCENTRATION AND VARIED CALCIUM

SERIES A. UNIFORM CALCIUM (249 P.P.M.), VARIED H-ION CONCENTRATION

pH VALUE	SEED			SEEDLINGS			
	SURVIVING TO EMERGENCE	NOT SURVIVING	SIGNIFICANCE BY χ^2 TEST	TOTAL OBSERVED	SURVIVING AT 18 DAYS	NOT SURVIVING	SIGNIFICANCE BY χ^2 TEST
	number	*number*		*number*	*number*	*number*	
4.82†	22	2	.....	22	14	8	.....
3.70	24	0	.....	24	18	6	.....
4.37	21	3	.....	21	13	8	.....
6.23	18	6	‡	18	4	14	§
7.53	18	6	‡	18	0	18	§

SERIES B. UNIFORM H-ION CONCENTRATION (PH 4.82), VARIED CALCIUM

CALCIUM LEVEL	SEED			SEEDLINGS			
	SURVIVING TO EMERGENCE	NOT SURVIVING	SIGNIFICANCE BY χ^2 TEST	TOTAL OBSERVED	SURVIVING AT 18 DAYS	NOT SURVIVING	SIGNIFICANCE BY χ^2 TEST
p.p.m.	*number*	*number*		*number*	*number*	*number*	
1×†	22	2	.....	22	14	8	.....
0×	22	2	.....	22	15	7	.....
5×	15	9	§	15	0	15	§
10×	14	10	§	14	0	14	§
15×	7	17	§	7	0	7	§

* Determined by YATE'S method (1, table VIII). ‡ = significant at the 5 per cent. level.

† Control hypothesis.

§ = highly significant at the 1 per cent. level.

Figure 1, B, represents results of tests in culture media with five levels of soluble calcium and a common pH level of 4.82. Responses of germinating seed and of seedlings in cultures containing no added calcium and

in cultures containing 249 parts per million of added calcium were closely parallel. In each of these pairs of cultures, 22 of the 24 planted seed germinated and 34 per cent. of the seedlings died. Chi-square tests indicated that behavior of seed and seedlings was not significantly different for these two cultures, but that the mortality in each of the three cultures with higher calcium was significantly greater than that in the control culture (table III, series B).

Relative osmotic pressures

In table IV are given the corrected freezing-point depressions and calculated osmotic pressures of (1) 11 culture solutions, containing 1 to 5 times as much calcium as the "balanced" solution; (2) a calcareous nursery soil solution; and (3) the expressed sap of the roots and tops of young seedlings grown in acid soil. The pressures for all the culture solutions are lower than that for the expressed sap of the roots, 5.30. The alkaline soil solution, with a pH value of 7.8, had an osmotic pressure of only 0.42, a value closely approximating those for agricultural soils. The osmotic pressures of expressed saps of roots and top have the general relation characteristic for most plants, a top-root ratio of more than 1:1.

TABLE IV

FREEZING-POINT DEPRESSION AND OSMOTIC PRESSURE OF CULTURE SOLUTIONS, HIGHLY CALCAREOUS SOIL, AND CELL SAP OF ROOTS AND TOPS OF YOUNG SHORTLEAF PINE SEEDLINGS

Material	Freezing-point depression (corrected)	Osmotic pressure
Culture solution	° *C.*	*atm.*
a. pH 4.8, Ca × 1	−0.265	3.19
b. pH 6.2, Ca × 1	−0.320	3.85
c. pH 4.8, Ca × 1.45	−0.330	3.97
d. pH 4.8, Ca × 1.90	−0.335	4.03
e. pH 4.8, Ca × 2.36	−0.340	4.09
f. pH 4.8, Ca × 2.81	−0.350	4.21
g. pH 4.8, Ca × 3.27	−0.355	4.27
h. pH 4.8, Ca × 3.72	−0.360	4.33
i. pH 4.8, Ca × 4.18	−0.360	4.33
j. pH 4.8, Ca × 4.63	−0.370	4.45
k. pH 4.8, Ca × 5.00	−0.380	4.57
Calcareous soil solution (pH 7.8)	−0.115	0.42
Expressed seedling sap		
Root	−0.440	5.30
Top	−0.810	9.75

None of the culture solutions listed in table IV plasmolyzed cells of sections of young seedling rootlets submersed in them for 2 hours. This was expected, on the basis of the relative osmotic pressures of expressed root sap and the solutions. After 48 hours, however, it was evident that germi-

nating seed on filter papers saturated with the solutions containing more calcium than the "balanced" solution had been injured.

Buffer capacities of expressed saps

The buffer capacities determined on expressed root saps are presented graphically in figures 2 and 3. Figure 2 shows the relative buffering against change in pH of lots of sap from young seedling roots grown in acid (pH 5.6) and alkaline (pH 7.1) nursery soils. The generally parallel curves with closely similar gradients to the left of the zero ordinate indicate that there was little difference in the buffering of the two samples of sap against an acid. The reading on the glass-electrode potentiometer at the point of 22 drops of added acid solution indicated a 0.3 greater change for the sap having the higher pH value. This difference may not be significant. Both saps appeared well buffered in contrast with distilled water. When titrated with the N/10 alkali, the saps responded not only more quickly but differently; 6 drops of the alkali produced a change in pH of 2.1 in the less acid sap, but a change of only 0.9 in the more acid sap. The divergence of the curves must be observed in the vertical direction of the double arrow. The flattening off of the curves at their outer extremities was probably caused by buffering effects of sodium salt accumulation.

It may be noted that the pH value of the sap from seedlings produced

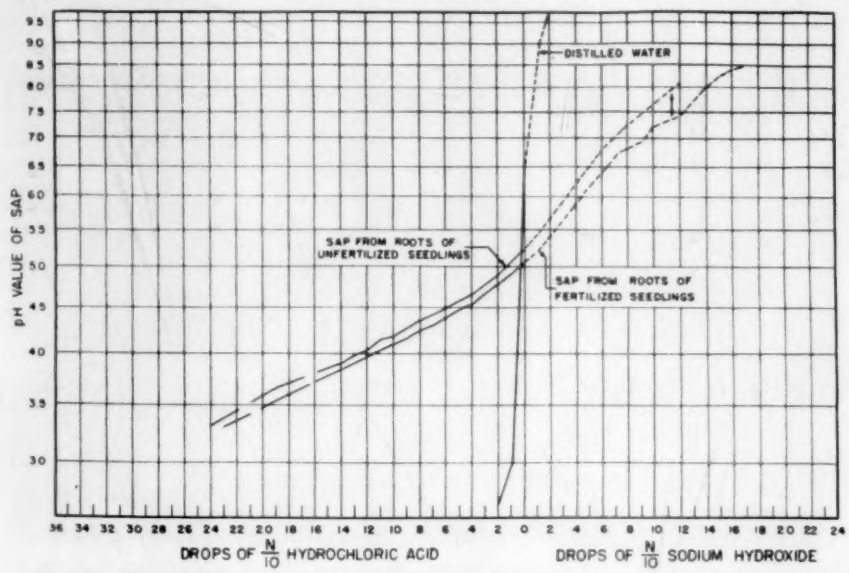

DISTILLED WATER
SAP FROM ROOTS OF UNFERTILIZED SEEDLINGS
SAP FROM ROOTS OF FERTILIZED SEEDLINGS
pH VALUE OF SAP
DROPS OF N/10 HYDROCHLORIC ACID
DROPS OF N/10 SODIUM HYDROXIDE

Fig. 3. Results of titrating cell sap expressed from roots of phosphorus-fertilized and unfertilized short-leaf pine seedlings against N/10 HCl and N/10 NaOH. In each case, the nursery soil had a pH value of 5.5. Trends of curves indicate relative buffering.

in alkaline soil was 5.4 and that of the sap from seedlings produced in acid soil was 4.8. While these two values have undoubtedly been affected in part by the corresponding soil-medium values, they do not equal the latter, which indicates a degree of buffering.

The buffer capacity of the cell sap of seedlings was somewhat increased by adding superphosphate to the soil. Colorimetric tests revealed a somewhat higher phosphorus content in the expressed sap of fertilized plants than in that of unfertilized plants. Little difference in buffering could be distinguished between the saps of fertilized and unfertilized seedlings when they were titrated against acid (fig. 3). A difference between these saps in buffering was indicated, however, when they were titrated against alkali. This differential is manifest in the pronounced divergence of the two graphs as viewed properly along lines parallel to the ordinate. Application of 12 drops of N/10 NaOH produced a change in pH value of +2.3 in sap from fertilized seedlings and one of +2.8 in sap from unfertilized seedlings, a difference of 0.5.

Water-soluble calcium

Water-soluble calcium tests indicated a high differential between soils, in two portions of the same nursery bed, which proved toxic and non-toxic to young seedlings developed from the same lot of seed (figs. 4 and 5). In the portion of the bed which produced thrifty seedlings (fig. 5), the surface inch of soil, with a pH value of 4.5, contained from 40 to 50 p.p.m. of water-

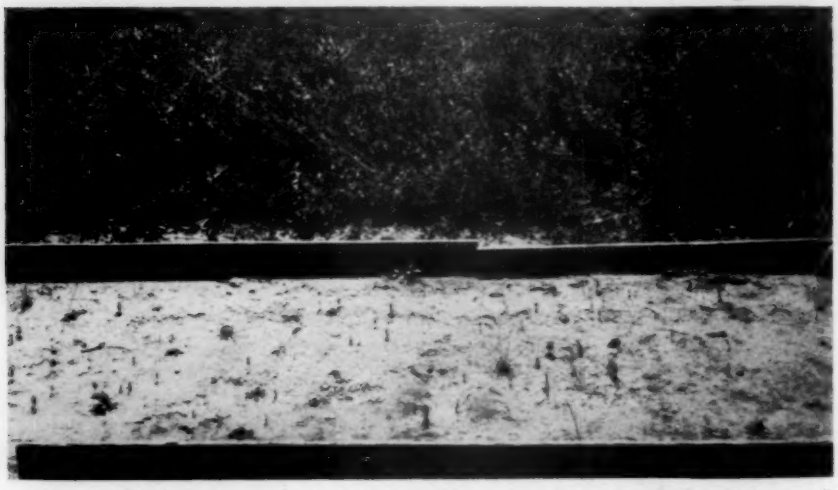


Fig. 4. Portion of nursery bed having 500 p.p.m. of water-soluble calcium and a pH of 7.8, in which shortleaf pine seedlings failed. In background, on similar soil, heavy growth of red clover.

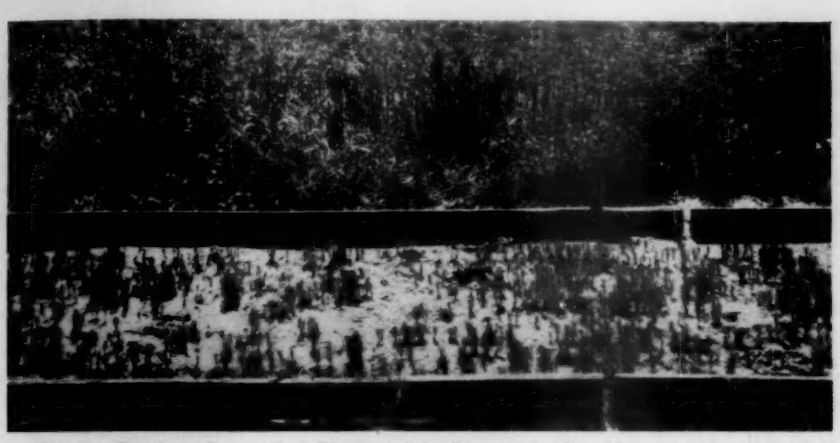


FIG. 5. Portion of same bed illustrated in figure 4 having 40 p.p.m. of water-soluble calcium and a pH of 5.4, in which shortleaf pine seedlings survived and developed well. In background, on similar soil, alsike clover.

soluble calcium. In a portion of the bed where more than 98 per cent. of the seedlings died soon after emergence (fig. 4), the surface inch of soil, with a pH value of 7.8, contained 500 p.p.m. of water-soluble calcium. A thicker, harder crust formed on the surface of the alkaline soil than on that of the acid soil. This crust was high in calcium; probably evaporation had effected a concentration of $Ca(HCO_3)_2$.

Determinations of the pH values of the soil in different portions of this nursery bed were confirmed by the behavior of red clover, *Trifolium pratense* L., and alsike clover, *T. hybridum* L., sown in mixture in an adjoining bed as a soiling crop. It is known that red clover develops well only on neutral or alkaline soils, and that alsike clover develops well on acid soils. On soil adjacent to that determined to have a pH of 7.8, a dense cover of red clover with little or no alsike developed (fig. 4); on soil adjacent to that determined to have a pH of 4.5, a less dense stand of alsike clover (fig. 5).

Discussion

The results of the present investigations contribute to isolation of the factors which confine the germination and survival of shortleaf pine to acid soil media low in soluble calcium. High soluble-calcium content and low hydrogen-ion concentration, or either one, may be lethal to germinating seed or young seedlings of this species (figs. 1 and 4, and table III). The effects of the factors studied are certainly direct physiological ones, not indirect ones brought about through creation of substratum conditions more favorable to damping-off fungi, since examination of the dying seedlings indicated no attack by parasitic fungi. This explanation is counter to that offered by ILLICK and AUGANBAUGH (5) for nonoccurrence of pitch pine on calcareous soils.

It appears improbable, because of the osmotic-pressure values determined (table IV) and of absence of flaccid cells from root sections submersed in culture solutions, that the injury took the form of plasmolysis of the root cells. This is particularly true for seedlings grown in nursery soil having an osmotic pressure of only 0.42 atmosphere. It is recognized that the osmotic pressure of cell sap may be affected by such factors as water content of cell and soluble organic and inorganic substances in the cell, which in turn vary with conditions of the substratum and with growth processes. The value (5.30) for the expressed root sap at the time of determination was not static, but subject to change within certain limits in the direction of change of solute content of the culture medium. MEYER and ANDERSON (**6**) recognized that "all species can become adjusted within limits to a change in the mineral salt content of the substratum. This adjustment takes the form of an increase in the osmotic pressure of the plant with an increase in the osmotic pressure of the medium from which it obtains its mineral salts." In view of such response of plants to osmotic-pressure changes in their culture media, it seems that shortleaf pine seedlings were in no danger of plasmolysis or physiological drought in culture solution "k" (table IV).

The pH values of the expressed saps of plants vary according to species, mostly between 3.0 and 7.0 according to MEYER and ANDERSON and others. For a particular species, the value may vary—within rather definite limits—from time to time and from one part of a plant to another without injurious effects. TRUOG and MEACHAM (**9**) and HAAS (**2**) found that the juices of most plants grown in unlimed soils were more acid than the juices of plants grown in limed soils. While the limits within which the pH values of sap of shortleaf pine seedling roots may fluctuate without injury have not been determined, it has been established that the values do vary with the acidity of the soil media.

It has been observed (fig. 1, and table III) that seedlings developed at pH 6.23 survive in significantly lower proportion than seedlings developed at lower pH values. Seedlings might be expected to tolerate a somewhat higher pH value in natural soil media than in culture solutions. No effort was made to determine the specific effect or effects of H-ion concentration in the culture solutions upon the cell protoplasm. (See MILLER (**7**), pp. 45–51, 262–265, and 1087–1088.) Of the many cell structures and processes known to be affected by H^+ and OH^- ions, it is not known which were adversely affected in these experiments when hydroxyl ions became toxic.

While a change in pH may effect a change in the relation of the elements in a soil medium which are essential for metabolic processes in seedling plants, there is little basis for belief that seedlings in the germination stage can be seriously affected by deficiency of minerals in the soil. Small quan-

tities of iron, phosphorus, and other basic ions present in soil media are often rendered unavailable by pH values above 6.5; but materials stored in the cotyledons are sufficient to support growth beyond the period in which most of the seedling deaths in these experiments took place.

The cytoplasm and cell sap of most plants, known to be buffered differently, are buffered within rather definite limits. It is recognized that the buffering of the expressed sap of shortleaf seedling roots (figs. 2 and 3) in general represents that of the root cells. The curves in figure 2 have two important features. First, both lots of expressed sap were much more highly buffered against the acid than against the alkali. (Cell saps are usually more highly buffered against one than against the other.) The saps were, in fact, buffered against a pH value lower than those found in many natural soils. Second, the sap of the roots from the acid soil was more highly buffered against alkali than the sap of the roots from the alkaline soil. This difference may be attributable to the smaller amount of phosphorus available in the latter soil, evidenced by colorimetric phosphorus tests on the two saps. The curves in figure 3 indicate that buffer capacity was somewhat increased by adding superphosphate to an acid nursery soil in which seedlings were developing; the difference might have been significantly greater if the superphosphate had been applied to an alkaline soil—a point for further study.

On the basis of theory, the explanation may be advanced that the toxic effect of excess calcium on the seedling root cells consists in a change in the permeability of the protoplasmic membranes, the degree of toxicity depending upon the ionic concentration of calcium. RABER (8) and others have expressed the theory that calcium at first decreases the permeability of these membranes and later increases it. In these experiments, increase in permeability may have been sufficient to result in free diffusion of substances in either direction through the cell membranes and finally, in culture media of high soluble-calcium content, in disorganization of the protoplasm in the root cells of seedlings.

The restrictions placed on seedling growth by calcareous soils may have a serious adverse effect on important phases of a forestation program. Shortleaf pine, if planted on old fields with high-calcium soils, may fail to perpetuate itself through natural regeneration. Unless nursery sites are judiciously selected on the basis of species tolerance for soil factors, costly soil-management practices become imperatively necessary for seedling production. As in the nursery beds used in these experiments (figs. 4 and 5), high water-soluble calcium is usually associated with high pH values, and low water-soluble calcium with low pH values. Reliable evidence of the viability of seed of shortleaf pine, necessary to production of high-quality nursery stock, can best be obtained by correlating seed responses with composition and condition of the germination media.

Summary

Germinating seed and young seedlings of shortleaf pine cannot survive in culture media having a soluble-calcium content of approximately 500 p.p.m. or more and a pH value of approximately 6.5 or more or having either of these characteristics. This was evidenced by behavior of seed in greenhouse cultures and of seedlings in nursery beds.

The osmotic pressures of the culture solutions and a solution of the alkaline nursery soil were not sufficiently high to effect plasmolysis of the seedling root cells.

Expressed sap of roots of seedlings a few weeks old was more highly buffered against an acid than against an alkali. Sap from roots developed in an acid soil was more highly buffered against the alkali than was that from roots developed in an alkaline soil. Buffering of the cell sap of roots may be somewhat increased by applying phosphorus to soils low in that element. The pH values of expressed root saps were lower in all instances than those of the soil media in which the roots developed.

Possibilities as to the nature of the toxic effects of high soluble-calcium and low hydrogen-ion concentrations on the root cells are briefly discussed.

High calcium content of the soil may prevent natural reproduction of planted shortleaf pine stands, increase the cost of soil management in forest nurseries, and render inapplicable the results of seed germination tests.

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THE EFFECT OF AERATION ON GROWTH OF THE TOMATO IN NUTRIENT SOLUTION[1]

WILLIAM DONALD DURELL

(WITH NINE FIGURES)

Introduction

It has long been recognized among plant physiologists that the air relations of roots have an extremely important bearing on both the vegetative and the reproductive phases of plant growth. It has been shown many times that on submerged soils and soils impermeable to air, most plants develop poorly or die early. The most common illustration of this fact is the "drowning out" of wheat in the Spring when water collects in small depressions of the ground.

The consensus of opinion among experimenters is that the air relations of roots have a direct influence on the absorption of water and on the absorption of nutrient ions from the soil solution, as well as on the more direct respiratory requirements of the roots as needed for the continual proliferation of new root tissue and root hairs.

Experimentation has shown that it is not the excess of water itself which is injurious, since plants develop perfectly in water cultures. It is, rather, a lack of aeration resulting from root-submersion which is harmful. Many plants grown in a nutrient solution will develop successfully only when the roots have direct access to sufficient oxygen in solution, as when the solution is thoroughly aerated, or when air is carried into the solution by means of continuous solution renewal.

SACHS (**12**), in his early work with nutrient solutions, discovered that the aeration of some of his cultures resulted in increased growth. After his work in 1860, the subject was given attention by various workers from 1901 to date, and it has been repeatedly shown that lack of aeration of the nutrient medium is an extremely important limiting factor in plant growth.

ARKER (**3**), working with lupines, found that root growth was accelerated by passing air through both soil and water cultures. HALL, BRENCHLEY, and UNDERWOOD (**8**), using lupines and barley, found that aeration of the nutrient medium resulted in a 50 per cent. increase in total dry weight of plants. PEMBER (**11**), found that barley plants did not respond to aeration when grown in solutions renewed periodically every two weeks. FREE (**7**), working with buckwheat in solutions which were renewed every two weeks found that bubbling air, oxygen, or nitrogen through the culture solutions produced neither beneficial nor injurious effects, but that the same treatment with carbon dioxide caused injury within a few hours, and death after a few days.

[1] This investigation was supported in part by a research grant from the Research Committee of the Graduate School, University of Kansas.

ALLISON (1) and ALLISON and SHIVE (2), showed that continuously renewed solutions produced soybean plants which were superior in all respects to those grown in periodically renewed solutions. Aeration of periodically renewed solutions resulted only in an increased root development. Aeration of continuously renewed solutions resulted in a marked increase of both tops and roots.

KNIGHT (9), using maize plants in soil cultures, found that aeration of the roots brought about a definite increase in dry weight. In water cultures, the maize plants failed to respond to aeration. It is noted here, however, that *Elodea canadensis* was used to aerate the solution, and that the quantity of oxygen liberated into the solution by this method may well have been too little to affect the growth of maize. Wallflowers and *Chenopodium album* on the other hand, showed considerable increase in dry weight when aerated by this method. KNIGHT also found that the root growth of maize was correlated inversely with the carbon dioxide content of the solution, rather than directly with the oxygen content.

CLARK and SHIVE (5), showed that aeration of continuously renewed solutions produced a marked increase in growth of both tops and roots of the tomato. The influence of aeration upon top growth was more pronounced than it was upon root growth. Although the plants in the non-aerated solution were much smaller, they started to blossom and fruit earlier than did those in aerated cultures. At the time of harvest (81 days) the aerated plants, however, showed evidence of yielding a much larger crop of fruit than the non-aerated plants.

LOEHWING (10), working with the sunflower and soybean in soil and sand cultures, found that aeration, providing less than 10 liters of air per kilogram of soil or sand per day caused early rapid growth and produced taller and heavier plants; it also resulted in larger root systems, more rapid nutrient absorption, and a much increased total weight. When more than 10 liters of air per kilogram of soil or sand was used, however, the plants were injured and retarded to a point even below that of the controls. This work suggests the possibility, under certain conditions, of reaching a point of excessive aeration which might have an adverse effect on plant growth. There is a possibility that LOEHWING's results might not apply to the aeration of a nutrient solution, where there would be no possibility of mechanical drying out of the roots.

ARRINGTON and SHIVE (4), using the tomato, showed that aeration of a continuously renewed nutrient solution produced a marked increase in the absorption rates of cation, anion, and total nitrogen over the corresponding rates from a non-aerated solution. Yields produced by aerated cultures were approximately double the yields produced by the non-aerated cultures. Carbon dioxide accumulation in the culture solutions was found to be with-

out effect on growth, rate of nitrogen absorption, or oxygen content of the solutions. This work of ARRINGTON and SHIVE demonstrated that lack of dissolved oxygen in the culture solution is a limiting factor in the growth of the tomato, rather than the carbon dioxide content, as suggested by FREE (**7**), and by KNIGHT (**9**).

It will be seen from this review of experiments on lupines, barley, buckwheat, soybeans, and tomatoes, that there is much evidence to indicate that aeration of the nutrient solution produces plants superior in vegetative growth to those grown in unaerated solutions. It is also apparent that past investigators have used a number of widely different methods for supplying the nutrient solution to the plant. Some of these methods have undoubtedly resulted in efficient aeration of the solution; others have resulted in varying degrees of insufficient aeration. These various methods might be outlined as follows:

1. Unaerated, unrenewed solutions, where the plant is allowed to complete its growth without the solution's being changed, renewed, agitated, or aerated.
2. Unaerated solutions replaced periodically by removing all the old solution and refilling to volume with new.
3. Unaerated solutions renewed periodically by adding new solution to volume.
4. Unaerated solutions renewed continuously with fresh solution, added by means of a drip.
5. Unaerated solutions renewed periodically by analysis and replacement of water and absorbed salts.
6. Unrenewed solutions aerated by bubbling air through the solution.
7. Periodically renewed solutions aerated by bubbling air through the solution.
8. Continuously renewed solutions aerated by air which is carried in along with the new solution.
9. Solutions periodically renewed but continuously circulated, and aerated by air which is forced in by the circulation mechanism.

As far as is known, no quantitative study has been made concerning the effects on plant growth of different degrees of aeration of the nutrient solution; nor has any optimum point been found, with regard to degree of aeration, for either vegetative growth or fruit production. As stated by ALLISON and SHIVE (**2**), it is impossible, on the basis of our present knowledge, to specify optimum conditions as regards oxygen requirements for plants in general, since these requirements have been shown to be distinctly variable among different species and even different varieties. It should be entirely possible, however, to specify through experimentation, the optimum conditions regarding oxygen requirements for a particular variety of plant in a given nutrient medium under controlled conditions.

It is evident that there is need for more knowledge concerning the effects of aeration on plant growth. Until we can discover the optimum air requirements of roots for certain plants under controlled conditions, and until we can standardize our treatment of such plants, our conclusions in the field of plant nutrition must necessarily be incomplete.

In view of the dearth of experimental information regarding quantitative aeration requirements of plants such as the tomato, and in view of the many existing commercial greenhouse installations for the use of nutrient solution cultures in producing crops of various plants, and of the distinct future commercial possibilities in this direction, it seemed advisable to set up an experiment with the following aims in view:

1. To determine, if possible, the optimum aeration for both vegetative and reproductive growth of the tomato plant.

2. To determine, if possible, the effect of varying amounts of aeration on total fruit production and on speed of fruit production as well as on dry weight of leaves, stems, and roots.

Procedure

The plant of the experiment involved a study of the tomato plant, variety Louisiana Red, as grown in nutrient solution, receiving five different treatments as regards aeration of the roots.

Seeds were planted in flats of clean sand, and the seedlings grown there for approximately three weeks, receiving frequent watering with the same nutrient solution that was used later in the experiment. When about 7 cm. high, the young plants were transferred to their permanent locations in the excelsior screens.

The nutrient solution used for all cultures was that found by SHIVE and ROBBINS (**13**), to produce excellent growth of tomatoes under average greenhouse conditions. It was composed as follows:

Salts	KH_2PO_4	$Ca(NO_3)_2 \cdot 4H_2O$	$MgSO_4 \cdot 7H_2O$	$(NH_4)_2SO_4$
Molar concentration	0.0023	0.0045	0.0023	0.0007

In order to supply the necessary trace elements, a supplementary solution which had proved beneficial in previous experiments at the University of Kansas was added to the nutrient solution in the following amounts:

Salts	H_3BO_3	$MnSO_4 \cdot 7H_2O$	$ZnSO_4 \cdot 7H_2O$	$CuSO_4 \cdot 5H_2O$
Parts per million	0.5	0.5	0.2	0.1

As a source of iron, iron tartrate in 0.5 per cent. solution was added regularly to the nutrient solution to give a concentration approximating 0.5 parts per million. Throughout the experiment, chlorosis was entirely prevented by the addition of iron tartrate in combination with carefully con-

trolled acidity of the solution. Daily titrations were made, and the hydrogen-ion concentration of the solutions was carefully kept within a range of pH 5.0 to 6.0, which was shown by CLARK and SHIVE (**6**), to include the point of maximum nitrate nitrogen absorption.

The culture tanks (4 feet by 10 feet by 6 inches) were made of heavy-gauge black iron, welded, and coated on the inside with hot asphalt.

Four snugly-fitting frames were constructed out of 2- by 4-inch lumber for each tank. One-inch-mesh iron wire screening was attached to the bottom of each frame, and the whole unit coated heavily with hot asphalt. Clean white pine excelsior was then placed in each screen to a depth of four inches to form a permeable supporting medium for the plants.

Asphalt covered heating cable was installed in the bottom of each tank and the entire installation regulated thermostatically to keep the solutions at a temperature ranging between 75° and 80° F.

Seedlings selected for uniformity were placed in the screens, sixteen to a tank, so that their roots were immersed in the solution which was two inches below the screen.

Tank no. 1 was filled with a mixture of one-third well-rotted cow manure and two-thirds sandy loam, as is practised in the commercial growing of tomatoes. This tank was furnished with several drainage outlets at the bottom, had no excelsior screen, and was watered regularly with water only. This tank was instituted here to compare the results of normal plant growth in soil with the others of the series.

Tank no. 2, in addition to the excelsior screen covering the solution, was fitted with a layer of heavy asphalt and sisal-bonded paper so that the plant stems projected through small holes. This was arranged in order to prevent, as much as possible, any diffusion of air into the solution. This treatment was instituted as a control to determine the results of growth in a solution lacking aeration.

All other treatments were set up to furnish varying degrees of aeration of the solution and to determine its effect on growth. In tank no. 3, as in nos. 4, 5, and 6, the excelsior screen was left uncovered and open to maximum diffusion of air through the screen into the solution.

Tank no. 4 was set up with a continuous drip-bubble apparatus after the method of SHIVE and STAHL (**14**). A 50-gallon accessory tank of nutrient solution was connected with a length of rubber tubing, and the solution fed into a Pyrex capillary feed-tube drop by drop, each drop carrying with it into the solution a definite amount of trapped air. At no time was there any overflow from this tank. A series of pinch-clamps regulated the flow of solution, and only enough was supplied to keep the level of the solution at a point corresponding with that of all other tanks. As the plants in this tank matured, it was necessary to increase the rate of flow of solution, and

consequently, the amount of air supplied to the solution. Weekly checks were made, however, on the amount of air supplied, and the calculation of 2.5 ml. of air per plant per minute for tank no. 4 is based on the average of these observations.

Tank no. 5 was furnished with a supply of compressed air giving 37.5 ml. of air per plant per minute. The air was supplied at this rate continuously, and was broken up by means of an aspirator into extremely small bubbles when making contact with the solution.

In tank no. 6, the solution level was slightly lower than in the others. The excess solution was led by gravity through an overflow pipe into a small covered tank. As the solution accumulated in this small tank, it actuated a centrifugal pump, thus pumping the excess solution into an overhead tank. From this tank, the solution flowed by gravity again into the main no. 6 tank, providing a continual circulation of the solution. The overhead tank supplied enough pressure that the solution could be squirted through Pyrex nozzles with some force into the main no. 6 tank in which the plants were growing. The average amount of air delivered into the solution, along with the returning solution, was calculated to be 250 ml. per plant per minute. The amounts of air used here were not planned as ideal quantities, but were rather the uncalculated results of efficiently functioning equipment. While not ideal for their purpose, it was felt that they would give useful indications of the effects of greatly differing amounts of aeration.

It should be noted here that the plants in all tanks but no. 1 received similar treatment except as to aeration of the nutrient medium. Daytime air temperature was kept between 65° and 70° F. and at night between 60° and 65° F. The temperature (75°–80° F.) of the nutrient medium was in all cases the same. All plants received the same solution and all received the same amount of new solution per plant per day, as well as the same amount of total solution per plant.

Tanks no. 2, 3, 5, and 6, were given a supply of new solution to volume every 48 hours. Tank no. 4 differed only in that it received its supply of new solution to volume continuously, drop by drop.

For purposes of clarity in discussing experimental results, use will be made of the following descriptive terms: Treatment no. 1 will designate treatment of plants growing in tank no. 1, treatment no. 2 will designate treatment of plants growing in tank no. 2, etc.

Results

The cultures described here were started on January 1, 1939, and all plants were harvested on May 21, 1939. Fruit was picked and weighed as it ripened, and an accurate account was kept of the daily production of plants receiving different treatments. Leaves and petioles fallen by abscis-

sion were kept at the base of each plant for inclusion in the harvest. The dry weights quoted were obtained by storing the material in a hot dry room for several months, then drying it further in a vacuum oven at 80°.

Fruit production

The average fruit production per plant for each treatment based on the unit of aeration is shown in figure 1.

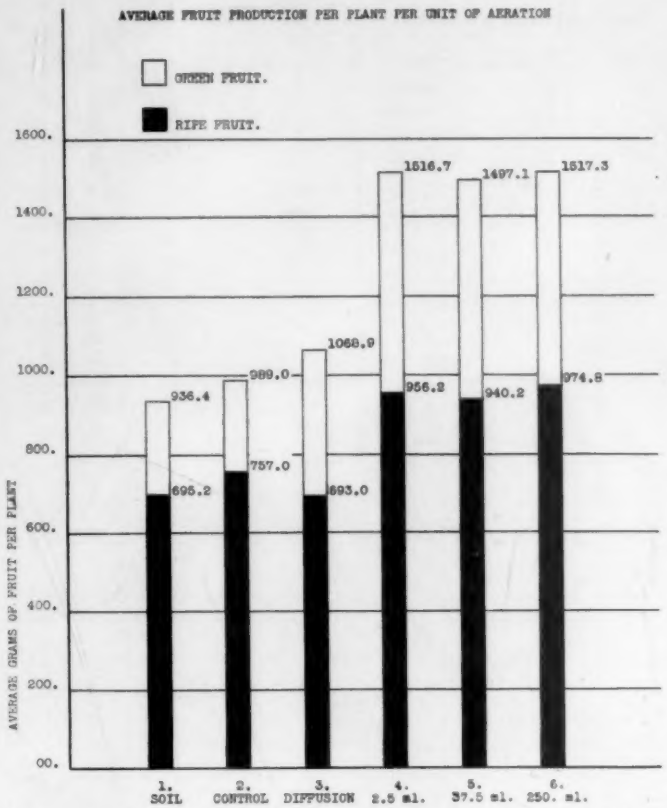

Fig. 1. Average fruit production per plant per unit of aeration.

The increase in both ripe fruit and total fruit production, due to aeration of the nutrient solution, is clearly illustrated by this table. The difference in results between treatments 3 and 4 is highly significant. The differences, however, between treatments 1, 2, and 3, and the differences between treatments 4, 5, and 6, are not considered significant, as the percentage variation is less than 10 per cent. The average speed of ripe fruit production per plant for each treatment, based on the number of days of growth, is shown in figure 2.

An interpretation of these results indicates that an extremely small amount of aeration of the nutrient medium, such as that of treatment no. 3, has a beneficial effect on the speed of ripe fruit production only in the early stages of growth. Plants receiving treatment no. 3, however, show a distinct lag in speed of ripe fruit production, especially in the later period of growth, behind plants given treatments no. 4, 5, and 6, all of which received a greater amount of air than those given treatment no. 3.

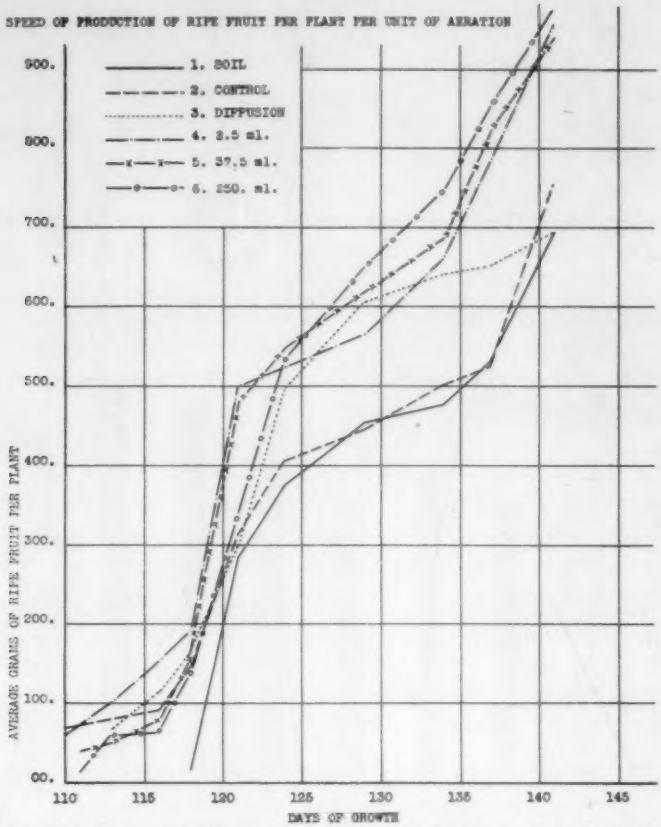

FIG. 2. Speed of production of ripe fruit per plant per unit of aeration.

On the other hand, a greater degree of aeration, as represented by treatments no. 4, 5, and 6, had a much more beneficial effect on both total fruit production and speed of ripe fruit production.

The most pronounced feature of these results, however, is the fact that the aeration requirements for optimum fruit production are low. Amounts of air greater than 2.5 ml. per plant per minute have very little effect on either total fruit production or speed of ripe fruit production. This can

perhaps best be noted in figure 3, where the results of treatments 2, 4, 5, and 6, supplying no air, 2.5 ml., 37.5 ml., and 250.0 ml. of air, respectively, per plant per minute are plotted in a curve based on amount of air per plant per minute.

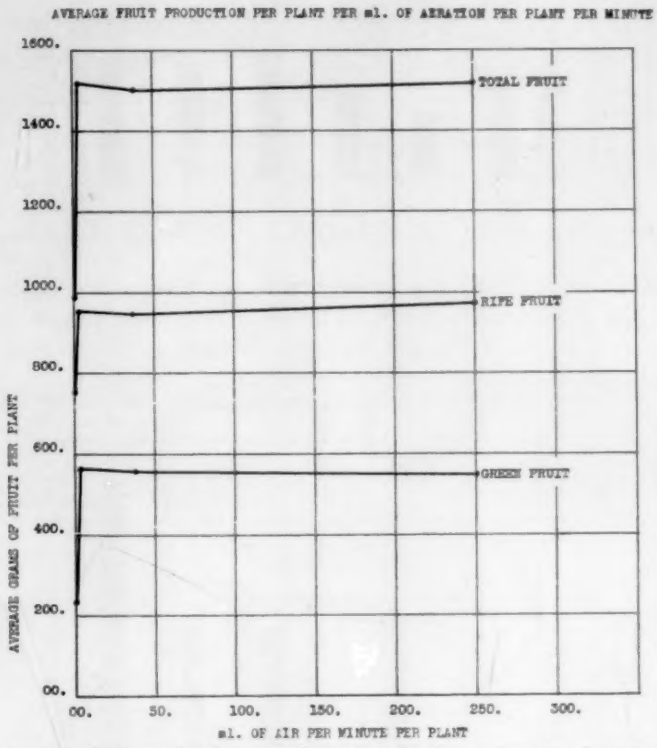

FIG. 3. Average fruit production per plant per ml. of aeration per plant per minute.

The slight superiority of treatment no. 2 over the soil grown plants of no. 1 is not significant; it might possibly be due to a stimulus of the reproductive phase of the plant, brought about by a lack of oxygen. This conclusion is apparently borne out by the fact that treatment no. 2 produced less vegetative growth than any one of the other treatments including no. 1.

VEGETATIVE GROWTH

The average dry weight of roots per plant, based on the unit of aeration is illustrated in figure 4.

The beneficial effect of air is clearly shown here by the fact that treatment no. 2, with no aeration, produced the smallest dry weight of roots. Treatments no. 1 (the soil grown plants) and no. 3 (receiving diffused air) each produced significantly larger quantities of roots than did treatment

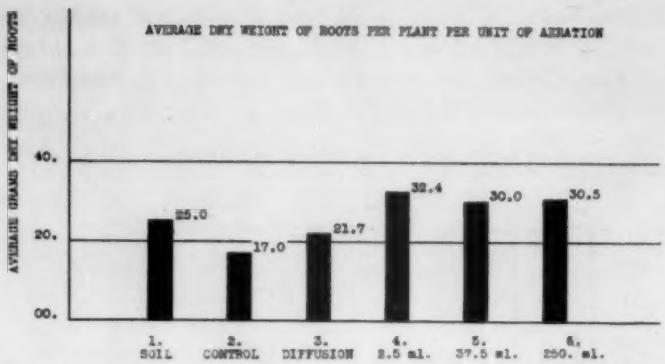

FIG. 4. Average dry weight of roots per plant per unit of aeration.

no. 2; treatments 4, 5, and 6, all receiving an aeration of more than 2.5 ml. per plant per minute each produced much larger quantities of roots than either no. 1, 2, or 3. These results indicate that, as in the case of fruit

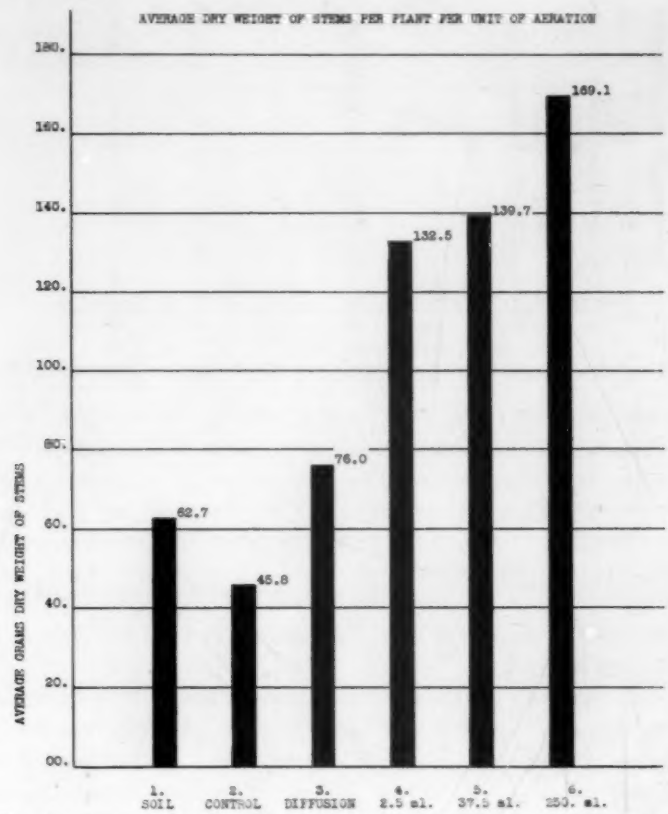

FIG. 5. Average dry weight of stems per plant per unit of aeration.

production, the air requirement for optimum root production is low; amounts of air over 2.5 ml. per plant per minute have very little effect on the production of roots.

Figures 5 and 6 show the average dry weight of stems and leaves per

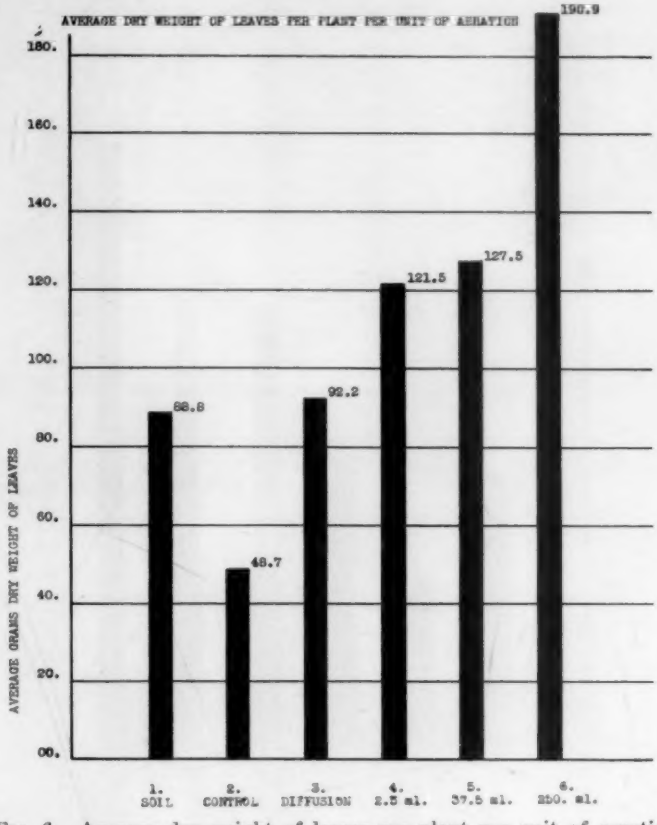

FIG. 6. Average dry weight of leaves per plant per unit of aeration.

plant, based on the unit of aeration. The beneficial effect of aeration is clearly shown in both tables. The smallest average weight of both stems and leaves was produced by plants receiving treatment no. 2, which provided no air, while the dry weights of plants receiving the other treatments are seen to exhibit a proportional response to the amount of aeration per plant per minute.

An interpretation of the results indicates that the aeration requirements for optimum stem and leaf production are high. This is shown by the fact that the greatest amount of both stems and leaves was produced by plants receiving treatment no. 6, which provided the greatest amount of air. It is

possible that the optimum amount of aeration for stem and leaf production was not reached in these experiments.

The average, total dry weight per plant including leaves, stems, and roots, based on the unit of aeration is illustrated in figure 7.

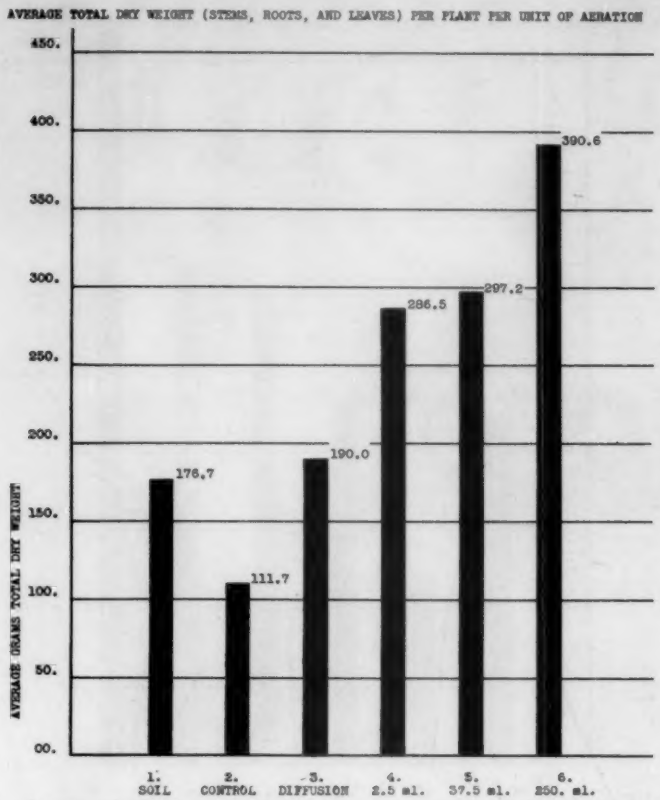

FIG. 7. Average total dry weight (stems, roots, and leaves) per plant per unit of aeration.

Figure 8 shows the effect of increasing amounts of aeration on total dry weight. The rising curve here again indicates, perhaps more clearly, the possibility that the optimum amount of aeration for total vegetative growth was not reached in these experiments. On the other hand, the marked increase in dry weight due to even a small amount of aeration is clearly shown.

No attempt was made to determine the various oxygen tensions of the solutions receiving different air treatments. As shown by figures 6, 7, and 8, however, it is possible that none of the cultures received the amount of dissolved oxygen in the solution which was necessary for optimum vegetative growth. The possibility is clear therefore, that none of the culture solutions was aerated to a point of oxygen saturation.

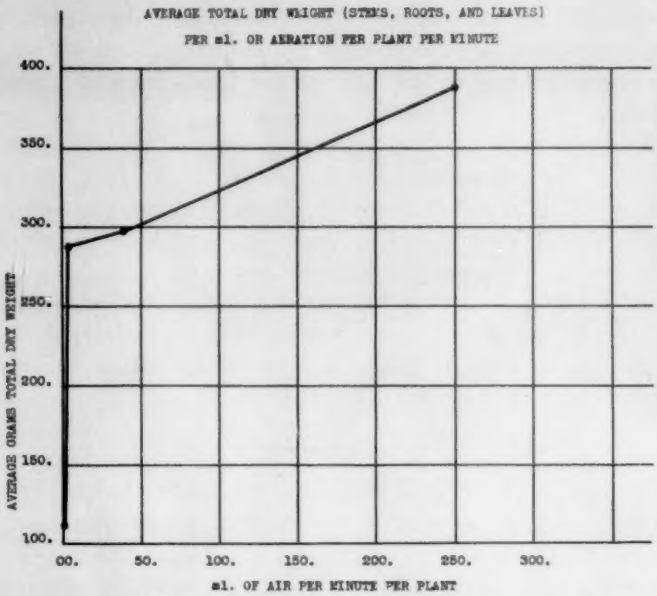

FIG. 8. Average total dry weight (stems, roots, and leaves) per ml. of aeration per plant per minute.

Several interesting observations were made during the course of the experiment.

The roots growing in tank no. 2, unaerated, tended to project their root-tips above the level of the solution, and to produce a large number of long-

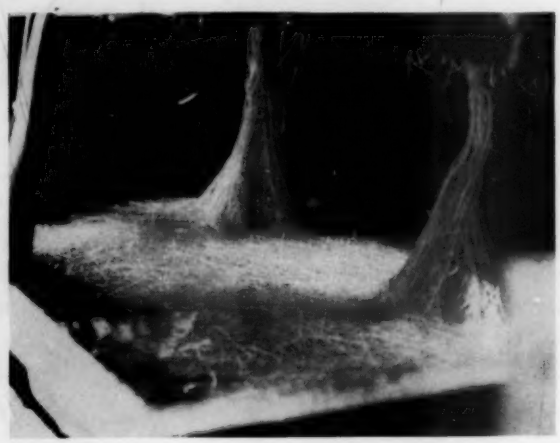

FIG. 9. Root system of mature plants grown in tank no. 2. Several root tips shown projecting above the surface of the solution.

lived root hairs in the damp atmosphere between the solution and the asphalt-paper covering. The roots of the aerated tanks, however, kept their root-tips below the level of the solution and produced a very small number of root-hairs.

The fruit produced by these plants was sampled by several persons, and no noticeable effect of aeration on the quality of the fruit was discovered. The fruit produced by solution-culture plants, however, was definitely firmer and meatier than that produced by the soil-grown plants. This observation was confirmed when some of the fruit was canned.

At the time the plants were harvested, plants receiving treatments number 1, 2, and 3, had slowed down considerably in their rate of vegetative growth; plants in tanks 4, 5, and 6, however, appeared to be continuing their vegetative growth at an unchanged rate.

Summary

In this paper a quantitative study was made of the effects of aeration of the nutrient solution, as related to the fruit production and vegetative growth of the tomato.

1. Aeration was shown to have a decidedly beneficial effect on the production of fruit, as well as on the vegetative growth of roots, stems, and leaves.

2. Aeration of the nutrient medium merely by natural diffusion of air had no significant effect on total fruit production or upon the speed of ripe fruit production, except in the early stages of growth.

3. Aeration of the nutrient medium by artificially supplying the solution with 2.5 ml. of air per plant per minute, or more, greatly increased both total fruit production and the speed of ripe fruit production.

4. Optimum fruit production was obtained when the nutrient solution was supplied with 2.5 ml. of air per plant per minute. Increasing the rate of aeration was without effect.

5. Optimum production of roots was obtained when the nutrient solution was supplied with 2.5 ml. of air per plant per minute. Increasing the rate of aeration was without effect.

6. Optimum production of stems and leaves was probably not obtained in this experiment. Stem and leaf production, within the limits of the experiment, are shown to be proportional to the rate of aeration; the greatest production was obtained with a supply of 250 ml. of air per plant per minute.

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LACTIC ACID FORMATION IN ALCOHOLIC FERMENTATION BY YEAST

LEONORA A. HOHL AND M. A. JOSLYN

(WITH FOUR FIGURES)

Introduction

The rôle of lactic acid in alcoholic fermentation has been of interest to all investigators who have concerned themselves with the chemistry of this process. PASTEUR (**25**) did not regard lactic acid as a product of normal alcoholic fermentations. His evidence indicated that its occurrence in fermented media was always associated with the presence of contaminating organisms. Subsequently, its occurrence in alcoholic fermentations, free of bacteria, was established, and several theories were proposed in which lactic acid played the rôle of a three-carbon intermediate between sugar and the end products, carbon dioxide and alcohol. BUCHNER and MEISENHEIMER (**3**) on the basis of their work with yeast press juice, first reported lactic acid to be such an intermediate; later, however, as a result of experiments made with pure yeast cultures, they concluded that live yeasts neither form nor ferment lactic acid. SLATOR (**29**) failed to agree with BUCHNER and MEISENHEIMER'S early work, considering lactic acid as a by-product rather than intermediate in alcoholic fermentation. The proponents of the WOHL-SCHADE (**6, 18, 26**) hypothesis held lactic acid to be an intermediate product in alcoholic fermentation. NEUBERG and TIR (**23**), FERNBACH and SCHOEN (**9, 10**), and EULER and LINDNER (**8**), also presented evidence for the intermediate rôle of lactic acid in alcoholic fermentation. Among those who could not confirm this evidence were KERB and ZECKENDORF (**16**), and KOSTYCHEV and FREY (**17**). OPPENHEIMER (**24**) considered lactic acid to be a by-product of alcoholic fermentation. His work indicates that either dihydroxyacetone or glyceric aldehyde (probably the latter), may be regarded as precursors of lactic acid. Meanwhile NEUBERG (**20, 21, 22**), presented evidence to show that methyl glyoxal was the precursor of lactic acid in both yeast fermentation and that brought about by animal tissues. He considered the formation of lactic acid from sugar quite probable. The foregoing as well as many other investigations (**27, 28**), of the rôle of lactic acid in alcoholic fermentation were conducted chiefly to determine the mechanism of its formation and cannot be cited as evidence of its occurrence as a by-product of normal alcoholic fermentation. The frequent occurrence of lactic acid bacteria in alcoholic fermentations makes many of the early investigations of doubtful value. Recent reports (**1, 5, 7, 13**) have postulated the formation of lactic acid as a by-product of alcoholic fermentation of sugar by pure yeasts. DURMISHIDZE (**5**) concludes that lactic acid is a

true by-product of alcoholic fermentation of sugars and is not formed from malic acid nor in significant amounts from the nitrogenous constituents of the medium. It is a constant by-product and formed to about the same extent by several strains of yeast examined.

The purpose of this investigation is to determine the course of lactic acid formation in relation to the utilization of sugar during alcoholic fermentation by pure yeasts and to determine the effect of some external conditions upon the total quantities of lactic acid formed by these yeasts.

Methods

Seven closely related strains of yeast were used in this investigation. They were all found to be strains of *Saccharomyces cereviseae* (HANSEN) STELLING-DEKKER, when classified according to STELLING-DEKKER (**30**). The strains designated as Burgundy and Champagne were originally from the collection of PACOTTET of France (**2**). Numbers 2338 and 2368 were obtained from the American Type Culture Collection in July, 1937. The former was labelled *Saccharomyces ellipsoideus*, and the latter *Saccharomyces cereviseae*. The Tokay strain was originally from California Fruit Industries, Ltd. Yeast number 66 was isolated by CRUESS (**4**) and the FREI BROTHERS' (FB) strain was isolated by the writers from a naturally fermenting vat of wine at Frei Brothers' Winery, Healdsburg, California, 1935 vintage.

The media used for the experiments included grape juice, wort, and an artificial medium which was a modification of WILLIAMS and SAUNDERS' (**31**) medium with the rare elements and asparagine replaced by a small amount of yeast extract. The composition of this artificial medium was 200 gm. dextrose, 3 gm. ammonium sulphate, 2 gm. potassium acid phosphate, 0.25 gm. calcium chloride ($CaCl_2 \cdot 2H_2O$), 0.25 gm. magnesium sulphate, and 30 ml. yeast extract (containing 0.25 per cent. nitrogen, and 1.5 per cent. total solids), per liter. The media were sterilized in flowing steam for a period of one hour on each of three successive days.

Yeast starters were generally prepared by transferring a loopful of yeast from a young stock agar slant to a small volume of the particular medium to be used in the experiment. This was transferred after two or three days to another portion of the same medium. After two more days the experimental media were inoculated, using sterile serological pipettes, with a volume of this second culture, amounting to one per cent. of the volume of the medium being inoculated. The fermentations were conducted at room temperature, 18–25° C., averaging 22° C. Most of the fermentations were made in duplicate. There was close agreement between duplicates and only the averages are included in the tables.

The total volatile acidity was determined by titrating in the hot, 100 ml.

of steam distillate from a 10-ml. sample. The results thus obtained are probably somewhat high, since variable amounts of lactic acid are volatile with steam. The pH was determined by the quinhydrone electrode. Sugar was determined by HASSID's modification of WHITMOYER's procedure (**14**). Total fixed acidity was determined by subtraction of total volatile acidity from total titratable acidity, the latter being determined by direct titration of a sample diluted with hot water. Lactic acid was determined by the method of FRIEDEMANN and GRAESER (**11**). After evaporating the alcohol, those samples containing less than 0.2 gm. sugar per 100 ml. of medium, were prepared for analysis by treatment with copper sulphate and calcium hydroxide suspension.[1] Aliquot portions of the filtrate were then oxidized and titrated as described by FRIEDEMANN. After removal of alcohol, those samples containing more than 0.2 gm. of sugar per 100 ml., were acidified (with 2 ml. of 1 + 4 H_2SO_4 for each 100 ml. of medium), and extracted with diethyl ether for fifty hours in a continuous liquid extractor. The excess ether was then distilled off, and the sample was made to the original volume in a volumetric flask of suitable size. These extracts were then treated exactly as were the other samples. This was necessary to avoid errors due to aldehyde formation on oxidation of malic, tartaric, and succinic acids (**11**).

Results and discussion

LACTIC ACID FORMATION DURING FERMENTATION OF GRAPE MEDIUM

Tall eight-liter bottles fitted with sampling tubes, were filled with six liters of Thompson Seedless grape juice, having 116 milli-mols (20.9 gm.) of total reducing sugar (as dextrose), 6.34 milli-equivalents of total acid, 3.73 milli-equivalents of tartrate, and 0.12 milli-equivalents of total volatile acid per 100 ml. The pH was 3.67. This medium did not contain any lactic acid but had a trace of formic acid. These were sterilized and individual bottles inoculated with the seven pure yeast strains. Samples of approximately 100 ml. were withdrawn from the fermentations at intervals determined by the relative rate of fermentation over a total period of 75 days. The yeasts were removed from the samples by centrifuging, and the samples were then frozen and stored at $-18°$ C. until the analyses could be made.[2] At the end of the experiment the yeast sediment was examined microscopically and both dilution and streak plates were made on suitable media to verify the purity of the cultures. No contaminants were found.

The data obtained for Frei Brothers' yeast are given in table I, and

[1] Preliminary experiments on known solutions showed that there was 95 per cent. recovery of lactic acid from samples thus treated.

[2] Before the total titratable acidity was determined the tartrates which had been precipitated as a result of freezing were brought back into solution by heating the samples in crown capped bottles and allowing them to cool again to room temperature.

TABLE I

FORMATION OF LACTIC ACID DURING ALCOHOLIC FERMENTATION OF GRAPE MEDIUM BY FREI BROTHERS' YEAST

TIME	SUGAR UTILIZED*	TOTAL VOLATILE ACID FORMED†	TOTAL FIXED ACID FORMED†	LACTIC ACID FORMED	RATIO: EQUIVALENT LACTIC ACID / EQUIVALENT VOLATILE ACID
	m. mols	*m. eq.*	*m. eq.*	*m. eq.*	
12 hr.	7	0.04	0.10	0.11	2.75
24 "	12	0.37	0.49	.....	.....
36 "	20	0.49	0.87	0.29	0.59
48 "	24	0.50	1.12	0.25	0.50
59 "	31	0.53	1.23	.....	.....
72 "	50	0.71	1.17	.....	.....
83 "	67	0.72	1.25	0.35	0.48
96 "	67	0.73	1.32	0.37	0.50
108 "	72	0.75	1.25	.....	.....
120 "	82	0.79	1.39	0.49	0.62
6 days	88	0.81	1.23	0.62	0.76
7 "	98	0.79	1.07	.....	.....
8 "	104	0.79	0.93	0.75	0.95
9 "	107	0.83	0.59	0.75	0.90
11 "	111	0.81	0.67	0.89	1.09
14 "	114	0.88	0.91	.....	.....
17 "	113	0.85	0.56	1.09	1.26
21 "	114	0.86	0.49	1.11	1.40
26 "	114	0.83	0.14	1.31	1.57
31 "	114	0.85	0.21	1.20	1.41
38 "	114	0.86	0.17	1.15	1.33
46 "	114	0.86	−0.04	1.10	1.27
60 "	115	0.86	−0.14	1.17	1.36
75 "	115	0.89	−0.23	1.27	1.42

* Milli-mols per 100 ml.
† Milli-equivalents per 100 ml.

those for yeast 2338 are graphically presented in figure 1. In table II the significant trends in the acid data for all seven strains are summarized. It is evident from these data that lactic acid, in contrast to volatile acid (acetic) was produced throughout the fermentation of the sugar, while volatile acid accumulated most rapidly during the fermentation of the first half of the sugar (**15**). This fact is reflected in the ratios of the respective acids formed to the sugar utilized. The ratio of volatile acid formed to sugar utilized rose to 0.03 during the first 24 hours of fermentation; during the ensuing 60 hours while the first half of the sugar was being fermented, the ratio dropped to 0.01. It thereafter dropped rather slowly, while the remaining sugar was being fermented, to 0.007, where it remained until the end of the experiment. The ratio of lactic acid formed to sugar utilized, on the other hand, attained its maximum of 0.015 after 24 hours and there-

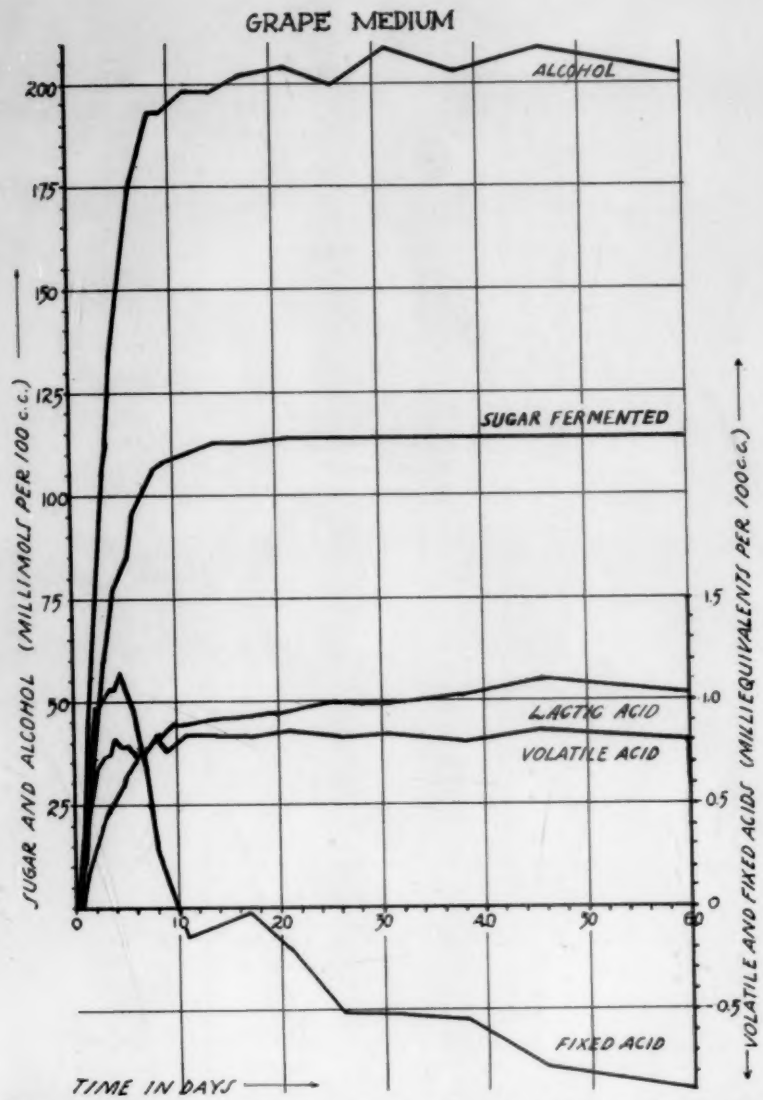

FIG. 1. Formation of lactic acid, volatile acid, and total fixed acid in relation to alcohol formation and sugar utilization in grape medium by yeast 2338.

after remained close to, or slightly below, 0.01. It thus shows a steady formation of lactic acid throughout the course of the fermentation. The same trend is reflected in the ratios of lactic acid to volatile acid formed at the various stages of fermentation.

In figure 2 the relationships between lactic acid formed and sugar uti-

TABLE II

COMPARISON OF ACID FORMATION BY THE SEVEN STRAINS OF YEAST DURING FERMENTATION OF GRAPE MEDIUM*

YEAST	SUGAR HALF FERMENTED			SUGAR COMPLETELY FERMENTED			FINAL SAMPLE		
	TOTAL VOLATILE ACID	TOTAL FIXED ACID	LACTIC ACID	TOTAL VOLATILE ACID	TOTAL FIXED ACID	LACTIC ACID	TOTAL VOLATILE ACID	TOTAL FIXED ACID	LACTIC ACID
	m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*
Burgundy	0.80	0.90	0.44	0.95	−0.07	1.42	0.92	−0.78	1.02
Frei Brothers'	0.71	1.25	0.35	0.83	0.67	0.89	0.89	−0.23	1.27
Tokay	0.81	1.23	0.66	0.84	−0.06	1.20	0.89	−0.87	0.90
2338	0.72	0.98	0.41	0.83	−0.19	0.99	0.84	−0.86	1.19
66	0.63	0.91	0.65	0.74	−0.04	1.07	0.75	−0.99	1.16
Champagne	1.17	0.61	0.66	1.30	−0.16	1.50	1.34	−0.84	1.13
2368	0.68	0.94	0.40	.....	.....	.....	0.87	−1.41	0.89

* Quantities expressed as milli-equivalents per 100 ml.

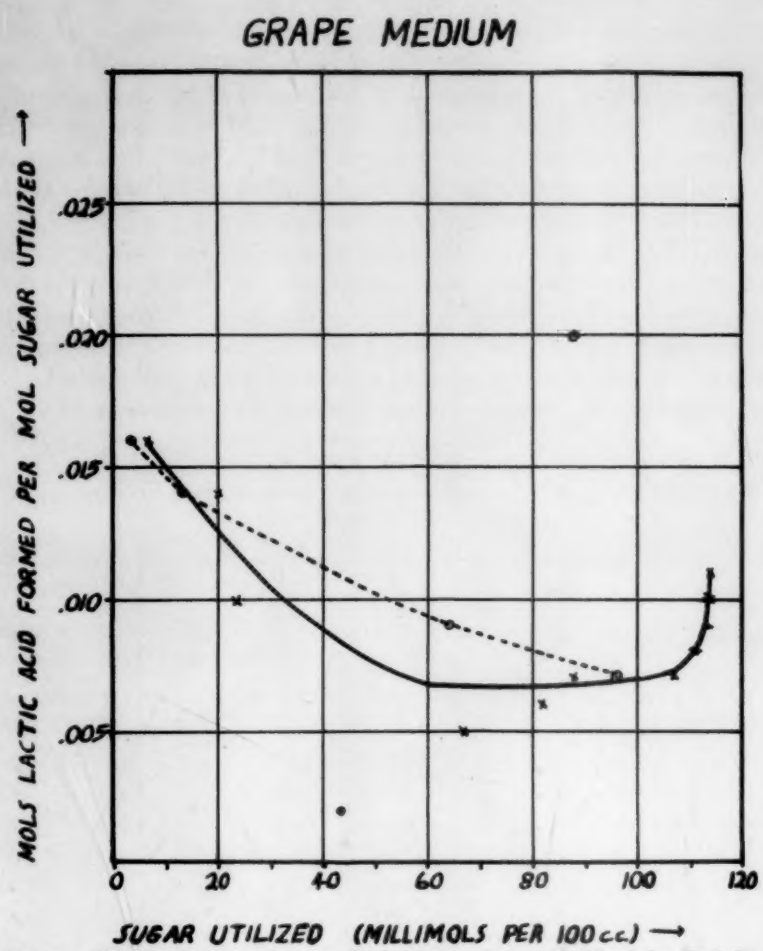

FIG. 2. Relation between lactic acid formed and sugar utilized by Frei Brothers' yeast in grape medium.

X = ratio of total mols lactic acid formed per total mols sugar utilized.

O = differential of increase in mols lactic acid formed to decrease in mols sugar utilized.

lized by Frei Brothers' yeast fermenting the grape medium are graphically presented. The solid line shows the ratio of total lactic acid formed to total sugar utilized, plotted against milli-mols sugar utilized. It may be seen that the ratio was relatively high at the beginning of fermentation and then dropped gently during the main course of the fermentation. During the final stages of the experiment after the sugar had all been utilized, lactic acid continued to be formed, thus producing a steep rise in the curve. This final rise in the curve indicates that in this stage, at least, lactic acid has

some precursor other than sugar; probably organic acid, such as tartaric acid, present in the grape juice medium. The broken line is interpolated among the calculated experimental points representing the ratio of the change in lactic acid to the change in sugar plotted against sugar utilized. This line was carried only to the completion of the sugar utilization since beyond this point it would approach infinity owing to the continued lactic acid formation after complete sugar utilization.

Tables I and II and figure 1 also show that the net total fixed acidity formed during the fermentation of grape juice by these yeasts reached a maximum when approximately half the sugar was fermented, and then dropped to about zero when the sugar was just fermented; it continued to drop thereafter to an average value of about minus one milli-equivalent per 100 ml. of medium at the end of fermentation. Determination of the tar-

TABLE III

FORMATION OF LACTIC ACID DURING ALCOHOLIC FERMENTATION OF ARTIFICIAL MEDIUM BY FREI BROTHERS' YEAST

TIME	SUGAR UTILIZED*	TOTAL VOLATILE ACID FORMED†	TOTAL FIXED ACID FORMED†	LACTIC ACID FORMED†	RATIO: EQUIVALENT LACTIC ACID / EQUIVALENT VOLATILE ACID
	m. mols	*m. eq.*	*m. eq.*	*m. eq.*	
24 hr.	5	0.08	0.02	0.05	0.61
48 "	9	0.26	0.10	.....	.....
60 "	11	0.35	0.21	0.06	0.17
72 "	12	0.43	0.31	.....	.....
84 "	15	0.48	0.60	.....	.....
96 "	17	0.55	0.61	0.15	0.27
108 "	18	0.63	0.65	.....	.....
120 "	20	0.67	0.59	.....	.....
6 days	26	0.83	0.57	0.18	0.22
7 "	33	1.04	0.64	0.33	0.31
8 "	39	1.12	0.52	0.41	0.36
9 "	47	1.31	0.41	.....	.....
10 "	51	1.43	0.61	.....	.....
11 "	58	1.52	0.90	0.52	0.34
12 "	69	1.58	1.08	.....	.....
13 "	71	1.68	0.94	.....	.....
14 "	76	1.70	0.92	.....	.....
16 "	85	1.85	1.55	.....	.....
18 "	93	1.86	1.76	0.69	0.37
20 "	100	1.98	1.42	0.73	0.37
23 "	102	1.91	1.55	1.12	0.58
27 "	103	2.00	1.86	.....	.....
32 "	104	1.87	1.69	.....	.....
38 "	104	1.91	1.63	1.29	0.67
45 "	104	1.96	1.28	1.38	0.70

* Milli-mols per 100 ml.
† Milli-equivalents per 100 ml.

taric acid and tartrates[3] present in the original and final samples showed that this net loss in fixed acid formation was due to utilization of tartrate as an accessory carbon source, which apparently started at an early stage of the fermentation. This utilization of tartrate was sufficient to mask the formation of lactic acid. Frei Brothers' yeast showed the lowest net loss of fixed acid but its lactic acid accumulation was typical of the group.

Table II shows that there were slight individual differences among the yeasts with respect to the quantities and rates of lactic acid formation in the grape medium; in general, however, this acid accumulated throughout the fermentation. With Burgundy, Tokay, and Champagne yeasts there is some evidence of utilization of lactic acid as a carbon source, after sugar had been depleted. MEISSNER (19) observed lactic acid destruction in wines free of sugar to be correlated with an increase in volatile acidity, but he was probably dealing with contaminated cultures. In these experiments there was no marked correlation of this nature.

The pH values remained fairly close to 3.5 throughout fermentation of the grape medium by all seven yeasts.

LACTIC ACID FORMATION DURING FERMENTATION OF ARTIFICIAL MEDIUM

The artificial medium having 106 milli-mols dextrose, 1.77 milli-equivalents total acid per 100 ml., no volatile acid, no lactic acid, and a pH of 4.38, was fermented with the seven yeasts and samples were withdrawn as in the previous experiment. In tables III and IV and in figures 3 and 4 the data are presented in a manner corresponding to that used for the data from the grape medium. In general, the quantities of lactic acid formed and the relation of these to the quantities of sugar utilized by the respective yeasts were very similar in this medium to those observed in the grape medium. The ratios of lactic acid to volatile acid formed were considerably lower in the fermentation of this medium than in that of the grape medium since the actual quantity of volatile acid formed was higher in the artificial medium than in the grape medium. The trend of these ratios, however, was quite comparable in both types of media. From figure 4 it can be seen that, following the initial high point, the ratio of lactic acid formed to sugar utilized remained fairly constant; when the sugar had all been utilized, it rose rapidly because of continued lactic acid formation. The ratio $\frac{\Delta \text{ lactic acid}}{\Delta \text{ sugar}}$ varied in a manner similar to that found in the grape medium. Total fixed acid formation in this medium continued to rise, though irregularly, throughout the entire period of the experiment.

The pH of this medium dropped within the first three or four days from

[3] These were determined by the official procedure of the Association of Official Agricultural Chemists (1935, p. 168,)

TABLE IV

COMPARISON OF ACID FORMATION BY THE SEVEN STRAINS OF YEAST DURING FERMENTATION OF ARTIFICIAL MEDIUM*

YEAST	SUGAR HALF FERMENTED			SUGAR COMPLETELY FERMENTED			FINAL SAMPLE		
	TOTAL VOLATILE ACID	TOTAL FIXED ACID	LACTIC ACID	TOTAL VOLATILE ACID	TOTAL FIXED ACID	LACTIC ACID	TOTAL VOLATILE ACID	TOTAL FIXED ACID	LACTIC ACID
	m. eq.	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*	*m. eq.*
Burgundy	1.22	0.88	0.73	1.57	1.51	1.37	1.57	1.83	1.20
Frei Brothers'	1.43	0.61	0.52	1.91	1.55	1.11	1.96	1.28	1.37
Tokay	1.20	0.70	0.71	1.75	1.77	1.21	1.65	1.15	1.15
2338	1.06	1.32	0.52	1.52	1.60	1.19	1.51	2.17	1.25
66	1.13	0.93	0.50	1.40	1.72	1.29	1.40	1.74	1.29
Champagne	1.38	0.86	0.30	1.62	1.56	0.78	1.62	1.70	0.88
2368	1.48	0.66	0.39	.....	.....	.....	1.82	1.30	0.97

* Amounts expressed as milli-equivalents formed per 100 ml.

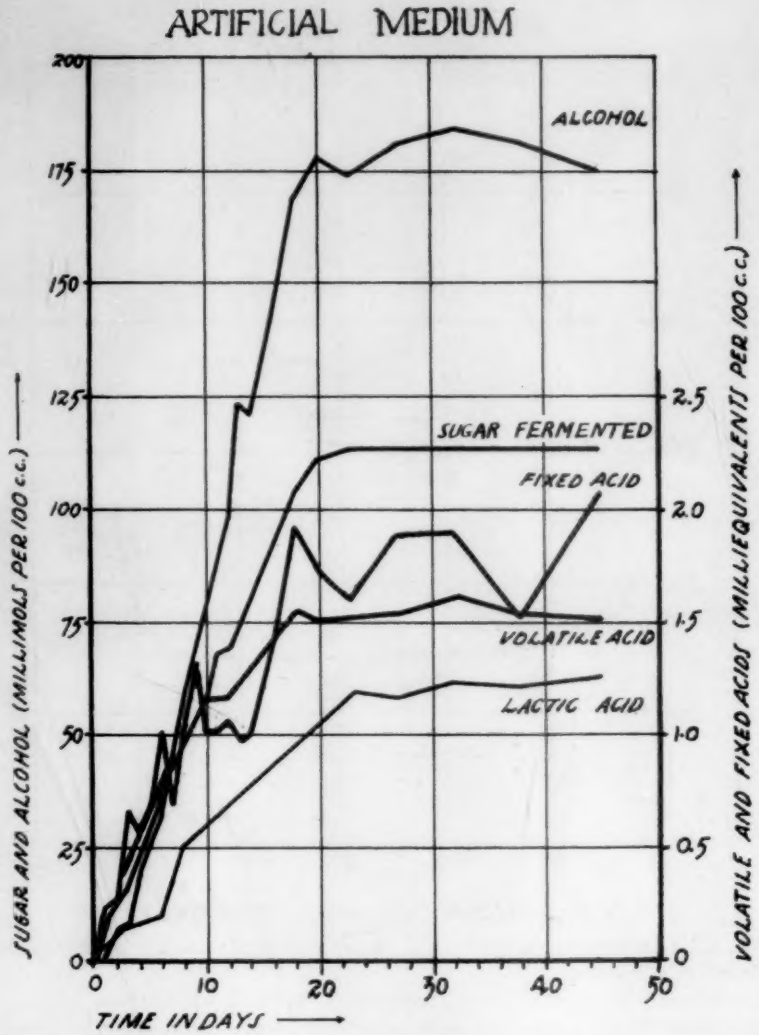

FIG. 3. Formation of lactic acid, volatile acid, and total fixed acid in relation to alcohol formation and sugar utilization in artificial medium by yeast 2338.

its initial value of 4.38 to 2.6–2.8, at which point it remained throughout the fermentation.

No very marked differences were noted among the seven strains with respect to amount and rate of lactic acid formation.

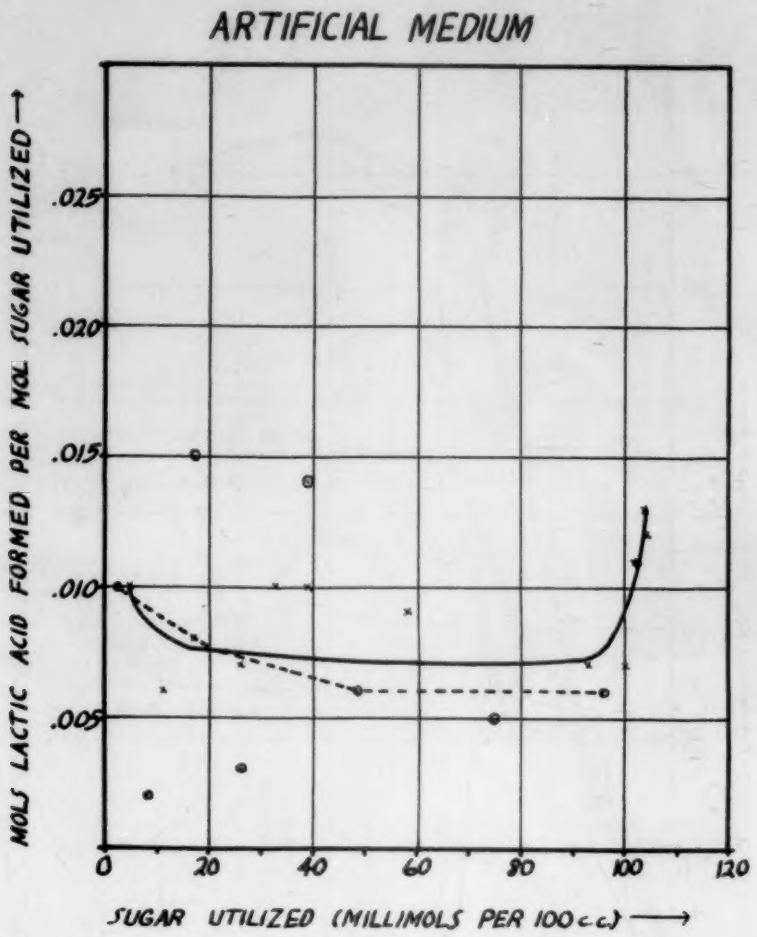

FIG. 4. Relation between lactic acid formed and sugar utilized by Frei Brothers' yeast in artificial medium.

X = ratio of total mols lactic acid formed per total mols sugar utilized.

O = differential of increase in mols lactic acid formed to decrease in mols sugar utilized.

EFFECT OF CARBON SOURCE AND NITROGEN SOURCE UPON LACTIC ACID FORMATION

To determine the effect of carbon source and nitrogen source upon lactic acid formation, several natural and artificial media were fermented with three of the yeasts (66, Champagne, and 2368). The natural media were white and red grape juice, diluted white grape concentrate, and unhopped beer wort. For the artificial media, the basic medium was modified. In

TABLE V

EFFECT OF CARBON AND NITROGEN SOURCE UPON LACTIC ACID PRODUCTION

CARBON SOURCE	NITROGEN SOURCE	YEAST 66			
		SUGAR UTILIZED*	LACTIC ACID FORMED†	TOTAL FIXED ACID FORMED†	RATIO: EQUIV. LACTIC ACID FORMED / MOLS SUGAR UTILIZED
		m. mols	*m. eq.*	*m. eq.*	
White grape juice	White grape juice	120	0.26	1.90	0.016
Red grape juice	Red grape juice	112	−0.71	0.95	0.008
Diluted grape concentrate	Diluted white grape concentrate	110	0.92	0.98	0.009
Wort	Wort	81	0.57	0.60	0.007
Cerelose	Liebig's beef extract	109	2.23	2.15	0.019
Cerelose	Peptone	110	1.49	1.25	0.011
Cerelose	Urea	110	1.41	1.39	0.012
Cerelose	Asparagine	111	1.16	1.45	0.013
Cerelose	l-Aspartic acid	110	−0.32	1.28	0.011
Cerelose	β-Alanine	14	1.50	0.23	0.016
Cerelose	dl-Alanine	110	2.01	1.39	0.013
Cerelose	dl-Leucine	110	1.73	1.53	0.014
Cerelose	Glycine	81	1.75	0.74	0.009
Cerelose	$(NH_4)_2SO_4$	95	1.56	0.90	0.009
Dextrose	$(NH_4)_2SO_4$	86	1.36	0.66	0.007
Levulose	$(NH_4)_2SO_4$	93	1.76	1.42	0.015
d-Galactose	$(NH_4)_2SO_4$	63	2.32	0.75	0.012
Maltose	$(NH_4)_2SO_4$	75	2.24	0.82	0.011
Sucrose	$(NH_4)_2SO_4$	97	1.82	0.90	0.009
		CHAMPAGNE YEAST			
White grape juice	White grape juice	120	0.58	1.85	0.015
Red grape juice	Red grape juice	112	−1.42	1.87	0.016
Diluted grape concentrate	Diluted white grape concentrate	110	0.38	1.31	0.012
Wort	Wort	81	0.37	0.42	0.005
Cerelose	Liebig's beef extract	91	1.90	1.95	0.021
Cerelose	Peptone	110	0.61	1.04	0.009
Cerelose	Urea	110	1.45	1.01	0.009
Cerelose	Asparagine	110	1.24	1.17	0.010
Cerelose	l-Aspartic acid	109	−0.17	1.50	0.014
Cerelose	β-Alanine	8	1.00	0.26	0.032
Cerelose	dl-Alanine	110	1.67	1.14	0.010
Cerelose	dl-Leucine	106	1.49	0.84	0.007
Cerelose	Glycine	50	3.74	0.27	0.005
Cerelose	$(NH_4)_2SO_4$	65	1.15	0.44	0.007
Dextrose	$(NH_4)_2SO_4$	80	1.42	0.60	0.007
Levulose	$(NH_4)_2SO_4$	89	1.59	0.71	0.008
d-Galactose	$(NH_4)_2SO_4$	3	.....	.....	.....
Maltose	$(NH_4)_2SO_4$	59	1.62	0.82	0.014
Sucrose	$(NH_4)_2SO_4$	65	1.26	0.53	0.008

TABLE V—(*Continued*)

Carbon source	Nitrogen source	Yeast 2368			
		Sugar utilized*	Total fixed acid formed†	Lactic acid formed†	Ratio: Equiv. lactic acid formed / Mols sugar utilized
		m. mols	*m. eq.*	*m. eq.*	
White grape juice	White grape juice	113	0.62	1.32	0.012
Red grape juice	Red grape juice	111	−1.29	1.13	0.010
Diluted grape concentrate	Diluted white grape concentrate	110	0.90	1.24	0.011
Wort	Wort	87	0.87	0.80	0.009
Cerelose	Liebig's beef extract	103	2.34	1.63	0.015
Cerelose	Peptone	107	0.28	0.77	0.007
Cerelose	Urea	80	0.81	0.36	0.004
Cerelose	Asparagine	82	0.71	0.75	0.009
Cerelose	l-Aspartic acid	80	−0.14	0.62	0.007
Cerelose	β-Alanine	11	1.28	0.28	0.025
Cerelose	dl-Alanine	75	1.64	0.66	0.008
Cerelose	dl-Leucine	84	1.50	0.65	0.007
Cerelose	Glycine	71	1.66	0.59	0.008
Cerelose	$(NH_4)_2SO_4$	50	1.00	0.60	0.012
Dextrose	$(NH_4)_2SO_4$	50	0.18	0.56	0.011
Levulose	$(NH_4)_2SO_4$	52	1.49	0.61	0.011
d-Galactose	$(NH_4)_2SO_4$	27	1.40	1.00	0.037
Maltose	$(NH_4)_2SO_4$	38	1.10	0.71	0.018
Sucrose	$(NH_4)_2SO_4$	48	0.17	0.50	0.010

* Milli-mols per 100 ml.
† Milli-equivalents per 100 ml.

one series the carbon source was always 200 gm. of cerelose (about 112 milli-mols dextrose) and the nitrogen source was varied so that the total amount of nitrogen added was always the same. In another series the nitrogen source was always three grams of ammonium sulphate per liter and the carbon source was varied, always using 200 gm. per liter, of the respective sugar. The sugar was determined in the initial and final media and was expressed, in all instances, as dextrose. Expressed in this manner, the initial sugar contents of this series of media were: dextrose 98, levulose 104, d-galactose 83, maltose 80, sucrose (inverted by acid hydrolysis) 117 milli-mols per 100 ml.

In table V the sugar utilized, total fixed acid, and lactic acid formed in the respective media are presented. A study of these data indicates that the amount of lactic acid formed by a given strain of yeast varies with the carbon source and with the nitrogen source. For a given medium there was also considerable variation in the amounts of lactic acid formed by the different organisms. The largest amounts of lactic acid per mol of sugar utilized were formed by all the organisms in the Liebig's beef medium, in

β-alanine, and by yeast 2368 in d-galactose. In the grape media the quantities of lactic acid formed were also fairly high.

In some of the fermentation, the "total fixed acid formed" was less than the lactic acid formed. This was probably due to utilization of some of the fixed acids.

Effect of oxygen supply upon lactic acid formation

Two series of the basic artificial media were fermented by the seven strains of yeast, using mercury fermentation bungs on one lot, and cotton plugs on another. These were fermented for two months with the results shown in table VI. The initial dextrose content of this medium was 110

TABLE VI

Effect of oxygen supply upon lactic acid formation

Oxygen supply	Yeast	Sugar utilized*	Lactic acid formed†	Ratio: Equivalent lactic acid / Mols sugar utilized
		m. mols	*m. eq.*	
Cotton plug	Burgundy	87	1.06	0.012
Hg bung	Burgundy	85	0.95	0.011
Cotton plug	Frei Brothers'	89	0.91	0.012
Hg bung	Frei Brothers'	90	0.90	0.010
Cotton plug	Tokay	91	1.12	0.012
Hg bung	Tokay	89	0.85	0.009
Cotton plug	2338	93	0.71	0.007
Hg bung	2338	94	0.76	0.008
Cotton plug	66	87	0.82	0.009
Hg bung	66	87	0.81	0.009
Cotton plug	Champagne	65	0.44	0.007
Hg bung	Champagne	78	0.80	0.010
Cotton plug	2368	50	0.56	0.011
Hg bung	2368	60	0.60	0.010

* Milli-mols per 100 ml.
† Milli-equivalents per 100 ml.

milli-mols per 100 ml. It may be noted that sugar utilization was incomplete in all of these fermentations. In general, the quantities of lactic acid formed by these seven yeasts varied so little under these two types of conditions, particularly in relation to the sugar utilized, that it may be concluded that formation of this acid is unaffected by the oxygen supply within these limits. Comparison of these data with those given in table IV for the final samples, indicates that a more liberal supply of oxygen might favor lactic acid production. The periodic withdrawal of samples from the fermentations shown in table IV brought about greater aeration than was possible in a fermentation which was simply cotton-stoppered and left undisturbed throughout its entire course.

The quantities of lactic acid found in this investigation are in close agreement with those reported by DURMISHIDZE (5) but they are about two to four times as great as those reported by ESAU (7) for yeast 66, during a ten-day fermentation period. ESAU's media contained 0.5 gm. of peptone and only 10 gm. dextrose per 100 ml. The amounts of lactic acid formed by yeast 66 in ESAU's experiments did not differ greatly in media buffered at pH 4.0, 6.0, and 8.0.

Of the seven yeasts studied here, Champagne yeast was the one which generally produced the least lactic acid, both in absolute amount and with respect to the sugar utilized.

Summary

1. The formation of lactic acid in the fermentation of natural and artificial media was investigated under various conditions.

2. During the course of fermentation of grape and artificial media, lactic acid accumulated at a relatively constant rate throughout the period of sugar utilization, indicating a close relationship between lactic acid formation and sugar utilization. The behavior of all seven organisms was very similar in these fermentations.

3. With a variety of carbon sources and nitrogen sources, the quantities of lactic acid formed were found to depend upon the composition of the media and upon the strain of yeast used.

4. No appreciable difference in lactic acid production was observed between fermentations with cotton stoppers and those with mercury bungs.

5. In general, these results confirm the earlier observations that lactic acid is an important by-product of alcoholic fermentation by pure yeast.

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PRESSURE–COMPOSITION RELATIONSHIPS OF THE GAS IN THE MARINE BROWN ALGA, *NEREOCYSTIS LUETKEANA*

GEORGE B. RIGG AND LYLE A. SWAIN

(WITH FIVE FIGURES)

Introduction

Analysis of the gas contained in the cavities of plants has attracted the attention of many investigators. Such analyses have usually been in relation to photosynthetic and respiratory exchanges, and therefore have considered only oxygen and carbon dioxide, residual gas being termed "nitrogen." The presence of 1 to 12 per cent. of carbon monoxide was reported in the marine brown alga *Nereocystis luetkeana* by LANGDON (**5**) and confirmed by LANGDON and GAILEY (**6**). This seems to be the first and only recorded instance of carbon monoxide being produced by a plant, which therefore merits further study, to which this paper is a contribution.

It is of interest to note that the presence of carbon monoxide was claimed in the blood of mammals, including man, in 1898 by SAINT-MARTIN (**20**) and by DESGREZ and NICLOUX (**1**), and in a series of papers by NICLOUX (**11, 12, 13, 14, 15**) who considered the carbon monoxide to be a normal product of metabolism. Several investigators have reported its presence in small quantities in human blood, including MCINTOSH (**10**), RATHERY *et al.* (**16, 17**), GETTLER and MATTICE (**4**), LOEPER *et al.* (**7, 8, 9**), ROFFO (**19**) and SCHMIDT (**21**).

Nereocystis luetkeana has been described by LANGDON but its main characteristics may be recapitulated. It is an annual found only in the sublittoral zone along the west coast of North America. The plant consists of a branched holdfast adhering to the rocks, a long, slender stipe extending from the holdfast to the water surface, and long, ribbon-like fronds attached to the free end of the stipe. The cylindrical stipe is the portion of particular interest. Its growth is very rapid (SHELDON) which results in the attainment of an extreme length of 20 to 25 meters in a single season. Its circumference gradually increases with distance from the holdfast, the stipe terminating in a bulb-shaped end 5 to 10 cm. in diameter. With this increase in circumference a longitudinal cavity develops in the center, leaving a surrounding wall 0.5 to 1.5 cm. in thickness. This hollow portion, or pneumatocyst, is buoyed up by the gas within it. The pressure and composition of this gas are the subject of the present study.

FRYE (**3**) found in the examination of a thousand specimens that the average pressure of the gas in the pneumatocyst was 77 mm. of mercury less than atmospheric pressure, the pressure ranging from 316 mm. less to 124 mm. greater. He showed that the pressure in a specimen was highest at 3–4 P.M. and lowest at 7 A.M., averaging 16 mm. difference.

Analysis of the gas in the cavity was first undertaken by ZELLER and NEIKIRK (**23**), who reported the presence of oxygen, carbon dioxide and nitrogen. Their work was not confirmed by LANGDON who analyzed the gas from a thousand specimens and found that it contained 1 to 12 per cent. carbon monoxide with an average of 4 per cent., and 15 to 24 per cent. oxygen with an average of 18 per cent., and occasional traces of carbon dioxide. He found no indication of hydrogen, methane, or ethylene. The presence of carbon monoxide was demonstrated by spectroscopic examination of blood exposed to the gas and by examination of the external and internal appearance of a guinea pig, a chicken, and a canary, all killed by inhaling the gas. In an experiment to investigate possible changes in the gas composition with the time of day, he collected 4 to 6 specimens at the end of each 4-hour interval, approximately, for a 24-hour period and analyzed their gaseous contents. The specimens of each group, however, showed so much difference among themselves that their average values were of no use in discovering any trend.

LANGDON and GAILEY concluded that the carbon monoxide was present as a result of the respiration of the plant. They found that it was produced in the absence of light; that if the gas in the cavity was replaced with air, carbon monoxide was redeveloped within a few days; and that if the cavity was filled with pure nitrogen or hydrogen, no carbon monoxide was produced. Further, a killed plant produced no carbon monoxide and autolysis of plant tissue produced only carbon dioxide and hydrogen.

RIGG and HENRY (**18**) found the inner surface of the pneumatocyst wall to be sterile, dispelling the possibility that the carbon monoxide might be of bacterial or fungal origin.

The work on Nereocystis reviewed above was done at the Oceanographic Laboratories (formerly the Biological Station) of the University of Washington at Friday Harbor, San Juan Island. The present investigation was carried out in the summers of 1937 and 1938 at the same location. It was planned to repeat some of the earlier work on Nereocystis, with the thought in mind that the changes in gas pressure shown by FRYE might be a reflection of changes in gas composition. As mentioned above, analyses of the gas from a small group of specimens collected at successive time intervals failed to test this hypothesis. The following experiments attempt to follow changes in gas composition by the analyses of a series of small samples taken from a given specimen at successive intervals of time.

Experimental

The apparatus used to follow the pressure variations was a modification of that described by FRYE. The essential part, of all-glass construction, shown in figure 1, was mounted on a board. The board in turn was mounted

on a sturdy raft 8 ft. by 10 ft. so that the apparatus could be moved vertically on slides, and clamped where desired, rigid with respect to the raft. Directly below the tip A (fig. 1), a specimen could be fastened firmly to the raft with the pneumatocyst in a horizontal position at the water level, where wave action kept it continually wet. Before a specimen was clamped in position it was loosened from the bottom to prevent entanglement of the stipe and anchor chain. Frequently the stipe broke when a plant was pulled up, but only those were used in which the break was close to the holdfast far below the beginning of the cavity so that there was no leakage in or out. As was shown by Miss FALLIS (**2**), Nereocystis is not affected by loosening the holdfast or breaking the stipe as long as the plant remains immersed in sea water. It was felt that this arrangement, when the raft was anchored in the harbor, retained the natural habitat of the plant.

FIG. 1. Mercury manometer.

With the specimen in place, the manometer was lowered, causing the perforated tip A to penetrate the wall of the cavity and the vaselined collar B of rubber pressure tubing, about 2 cm. from the tip, to form a seal which remained airtight for several days. The difference between atmospheric pressure and the pressure in the pneumatocyst was thereby immediately and continuously registered and was observed at frequent intervals. The temperature of the bathing sea water was noted at the same time. The atmospheric pressure at the time of observation was taken from a recording aneroid barometer, checked weekly with a calibrated cistern barometer. The absolute internal pressure in mm. of mercury was thus readily determined.

Successive samples of gas were satisfactorily withdrawn from a given specimen by the use of a 25-ml. hypodermic syringe lubricated with Lubriseal. The needle, filled with distilled water to prevent air-contamination of the sample, was inserted into the plant cavity through a thick layer of vaseline stiffened with 10 per cent. beeswax. A water drop was expelled within the cavity to clear the clogged needle tip, and then a 15-ml. gas sample was withdrawn. On removal of the instrument from the tissue, care was taken to seal the needle tip and the wound through the cavity wall by a slight lateral motion of the needle in the vaseline-beeswax mixture, temporarily softened by means of a hot glass rod. As a further precaution against contamination of the sample from the air, immediately after sealing the needle tip the pressure on the sample was increased to slightly greater than atmospheric. The gas sample was then transferred to an analytical apparatus, designed by GOODMAN and SWAIN (unpublished), by release under mercury.

Vaseline does not stick to the surface of the plant because of its slimy secretion of algin. It was found that heating the surface with an alcohol lamp caused it to remain dry and enabled the vaseline to adhere readily. Examination of the heated tissue after several days showed that the green discoloration (due to destruction of the brown pigment fucoxanthin, leaving the chlorophyll apparent) extended inward about 2 to 3 mm.; the remaining centimeter of tissue was brown and firm and appeared unharmed.

Similar observations on gas pressure and composition were made in the laboratory on specimens placed in a large tank of circulating sea water under controlled light intensities.

Results

The internal pressure was found to be less than atmospheric in the 52 specimens examined in the two summers, varying from 511 mm. to 713 mm. of mercury absolute pressure with an average of 604 mm., all uncorrected for temperature, which ranged from 10° to 14° C. The pressures found by FRYE lie within the limits of 444 mm. and 884 mm. absolute pressure with an average of 699 mm., on the assumption of an atmospheric pressure con-

stant at 760 mm. His values exhibit a similar but wider range than do those reported here, as might be expected from the smaller sampling used in the present study. They differ in that pressures exceeding atmospheric were recorded. Again confirming FRYE's observations, the pressure in any one specimen was found to be lowest in the morning, increasing during the day to a maximum value in the afternoon, and decreasing during the night. Figure 2 shows several typical curves obtained by plotting the absolute internal pressure, calculated to constant temperature, against time. FRYE suggested that this pressure variation might be caused by temperature fluctuations of the sea water. While it is true that the water is somewhat warmer during the day than at night, this merely accentuates slightly the pressure change, which figure 2 clearly shows, is a characteristic of the plant itself.

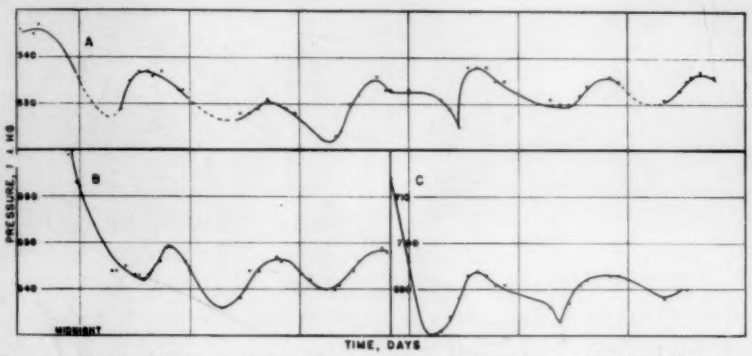

FIG. 2. Daily changes in internal pressure in Nereocystis. Vertical lines represent successive midnights.

The nightly decrease in gas pressure suggested an experiment to determine the effect of continuous darkness. In specimens placed in the laboratory tank of sea water in a dark room for several days, the pressure decreased throughout the time of the experiment. Typical results are shown in A and C of figure 3.

Since the pressure in the plant is different from that of the atmosphere, it follows that the pneumatocyst tissue is comparatively impervious to air. This was demonstrated in the laboratory by evacuating the bulb-shaped end cut from a pneumatocyst. A vacuum could be held as long as 24 hours before softening of the tissue at the cut surface caused leakage. When carbon dioxide was introduced into a fresh, evacuated bulb the pressure decreased rapidly as shown in B of figure 3, indicating the removal of the gas from the interior of the plant. LANGDON and GAILEY noted a similar reduction in pressure when hydrogen was introduced.

Analysis of samples of gas taken from a specimen immediately after

inserting the manometer showed a range of 17.8 to 24.6 per cent. oxygen, 0.8 to 7.6 per cent. carbon monoxide and 0 to 0.3 per cent. carbon dioxide (4 of 7 specimens so examined contained no carbon dioxide). These results confirm LANGDON's data except for the increased frequency of small amounts of carbon dioxide. In table I are given the results of the analysis of samples of gas removed over a period of time from three specimens exposed to different light conditions. It is readily seen that in specimen A, kept on the raft,

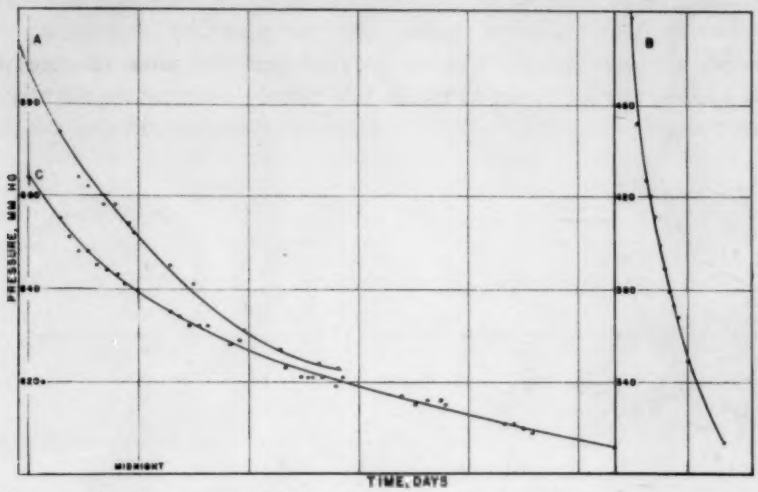

FIG. 3. A and C. Pressure change in Nereocystis in darkness. B. Pressure change in Nereocystis bulb containing only carbon dioxide.

the oxygen concentration increased during the day and decreased during the night, while the carbon dioxide behaved in the reverse direction. In specimen C, kept in the salt water tank in the dark room, the concentration of oxygen steadily decreased and that of carbon dioxide increased. Under a constant illumination of 300 foot-candles, obtained by two 200-watt electric lights in the dark room, specimen B behaved as though it were in total darkness.

These results are also presented in figure 4, accompanied by the pressure curve of each specimen. The vertical lines in the pressure curve represent the decrease in pressure resulting from the removal of a sample of gas. In one case in specimen A it represents an increase in pressure due to leakage of air into the plant when the sample was taken. Each gas curve indicates the change in quantity of that gas in the plant from immediately after a sample was removed until the next was taken, expressed in milliliters. For example, when the first sample was taken from A, the gas contained 20.62 per cent. oxygen, the pressure after removal of the sample was 634 mm. mercury, and the temperature was 11.6° C., from which it is found that the

specimen at that time contained 151.1 ml. of oxygen at standard conditions. When the second sample of gas was taken, the pressure was 639 mm., the temperature was 11.2° C., and the gas contained 21.22 per cent. oxygen, which represents 157.0 ml. oxygen at standard conditions. The oxygen curve shows this increase in content of 5.9 ml. In both specimens A and C the volume of the third sample removed was estimated, and the volume of entering air was calculated, to determine the changes in quantity of the sev-

TABLE I

CHANGES IN GAS COMPOSITION IN NEREOCYSTIS AT SEVERAL LIGHT INTENSITIES

SPECIMEN	DATE IN AUG. 1938	TIME	CO_2	O_2	CO
			%	%	%
A Daylight. Internal volume = 930 ml.	8	9:30	0.59*	20.62	1.35
		16	0.35	21.22	1.45
	9	7	0.54	19.76	1.00
		17	0.04	20.83	1.33
	10	8	0.72	19.78	1.39
		16:30	0.21	20.58	1.30
B 300 ft.-candles. Internal volume = 1080 ml.	6	19	0.00	22.70	0.00
	7	8	0.58	20.34	0.34
		17:30	1.11	19.00	0.11
	8	8	1.02	17.49	0.33
		19	1.23	16.29	0.39
	9	10:30	1.27	15.06	0.36
C Darkness. Internal volume = 610 ml.	1	16:30	0.00	17.84	1.74
		23	0.68	16.76	1.42
	2	7	1.16	14.69	1.89
		16:30	1.44	12.73	1.78
	3	9	1.71	9.69	2.02

* Sample removed 14 hours after the pressure manometer was inserted in the specimen.

eral gases. In C the volume of entering air equalled the volume of the sample removed.

The pressure curve of A shows an increase during the day and a decrease during the night, agreeing with the results shown in figure 2. The quantity of carbon dioxide increases during the night and decreases during the day, but not in sufficient amount to compensate for the opposite change in oxygen content. In B and C, in weak light and darkness, respectively, the pressure decreases continuously as does the oxygen content. The carbon dioxide content increases, but at a much slower rate than the oxygen decreases.

In all specimens, the quantity of carbon monoxide remained the same, within experimental error.

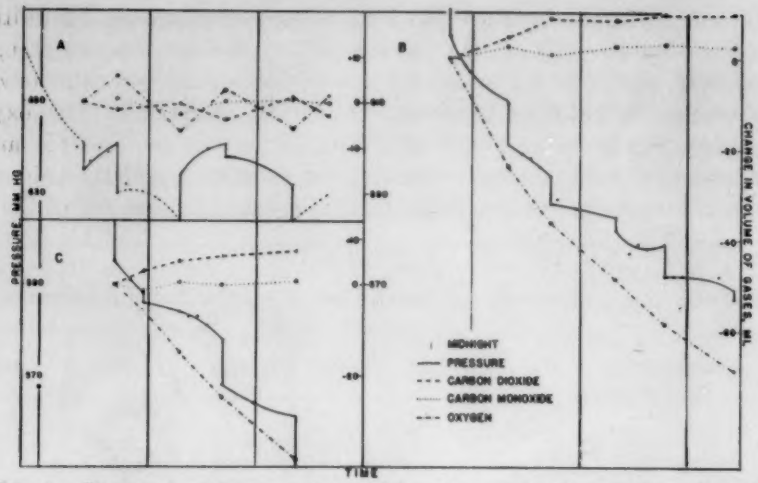

FIG. 4. Changes in pressure and quantities of gases in Nereocystis. A. On raft; B. 300 ft.-candles; C. Darkness.

Discussion

The foregoing results suggest the following hypothesis: The changes in internal pressure are a reflection of changes in the composition of the gas within the plant. Of the gases present, carbon dioxide occurs infrequently and then in very small amounts, the carbon monoxide content seems to remain constant (as shown in fig. 4), and nitrogen is not affected by respiration or photosynthesis. The changes in pressure therefore reflect changes in the amount of oxygen. The removal of oxygen from the specimens in darkness or weak light by respiration, possibly accentuated by injury, parallels the observed decrease in pressure. This decrease in oxygen content in Nereocystis in darkness corroborates LANGDON and GAILEY who showed that the oxygen content of uninjured plants kept several days in light-proof boxes in the harbor was far below the average value. Under normal conditions the decrease in oxygen content at night, caused by respiration, is accompanied by a decrease in pressure, and the increase in the amount of oxygen during daylight hours, caused by photosynthesis, is accompanied by an increase in pressure. Apparently the oxygen is used up in respiration and no corresponding volume of free gas is produced in the pneumatocyst. This might be due to the use of some compound of low oxygen content as respiratory material or to the absorption of the produced gas by the tissue of the pneumatocyst wall.

The development of carbon dioxide in all specimens under extended observation is possibly caused by injury to the plant by the clamps, the tip of the manometer, the needle of the syringe, or the heating necessary to

make the vaseline adhere. LANGDON and GAILEY showed, however, that it developed in their light-proof boxes. It is possible that excessive amounts of carbon dioxide cannot be readily removed from the gas space by the plant. In any event its frequent absence in the normal plant makes inseparable the changes in gas pressure and the oxygen content.

The rapid growth of Nereocystis, considered in relation to the impermeability of the tissue to air, suggests that the observed pressure is below atmospheric because air cannot enter the pneumatocyst cavity sufficiently rapidly to maintain atmospheric pressure. An observation giving support to this idea was made on *Macrocystis pyrifera*, growing in Neah Bay, Washington. This is a large brown alga closely related to Nereocystis. It consists of a holdfast and several long, cord-like stipes, to which are attached large broadly lanceolate fronds. At the base of each frond is a hollow cyst, corresponding to the pneumatocyst of Nereocystis. Growth occurs at the free end of the stipe, where new fronds and cysts develop.

Pressure in each cavity on several stipes was determined using an apparatus similar to that shown in figure 1, but constructed on a much smaller scale of fine bore capillary glass tubing. The results are shown in figure 5,

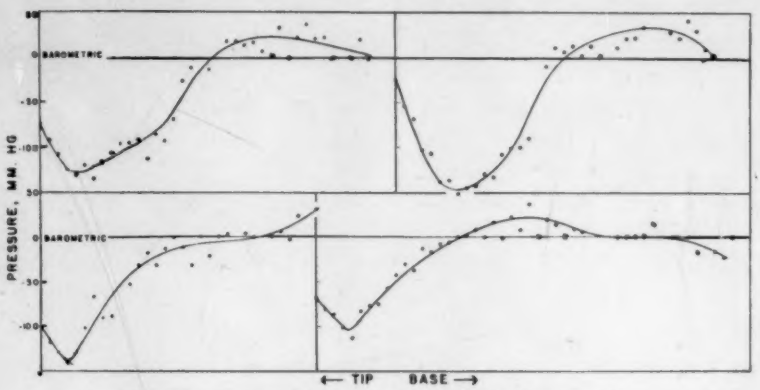

FIG. 5. Pressure in successive cysts of Macrocystis.

in which the pressure in each cavity, commencing at the free end, is designated by a point. Omission of a point indicates a broken cyst. It is evident that the region of rapid growth is characterized by pressures lower than atmospheric, whereas the pressures in the older, more mature region exceed atmospheric. All the specimens of Nereocystis observed in this study were vigorously growing plants.

Summary

1. The composition and pressure of the gas in the pneumatocyst of the marine brown alga, *Nereocystis luetkeana*, have been studied on single specimens for several days.

2. The daily change in pressure, from a maximum in the afternoon to a minimum in the morning, is confirmed.

3. The presence of oxygen and carbon monoxide and frequent absence of carbon dioxide (in normal specimens) are confirmed.

4. In plants in the sea the oxygen content increased during the day and decreased at night, corresponding with the pressure change.

5. In plants maintained in darkness, there was a continuous decrease in pressure and in oxygen content and a development of carbon dioxide.

6. The hypothesis is advanced that the change in pressure of the gas is caused by the change in its oxygen content.

7. From a brief study of the pressure in the cysts of *Macrocystis pyrifera*, the suggestion is made that the reduced pressures observed in both it and Nereocystis are the result of their rapid growth.

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X-RAY EFFECTS ON THE GROWTH AND REPRODUCTION OF WHEAT

D. J. WORT

(WITH FOUR FIGURES)

Introduction

Evidences of modification and injury to plant parts are numerous in the extensive literature on the effects of x-ray treatment of plants. JOHNSON (**2**) lists the effects produced on seventy species of flowering plants. Her work with wheat (**3**) shows that growing material is more strongly affected than non-growing by equal doses of radiation given in the same way. Seedlings from soaked grains given treatments of 1000 r-units and 5000 r-units made less growth in all respects than did controls, but showed increased tillering. No flowering data were included.

SHULL and MITCHELL (**4**) obtained deleterious effects when unfiltered radiations were used but the use of metallic screens, high voltage, low amperage, and brief exposures giving approximately 100 r-units, resulted in stimulative action on wheat, corn, oats, and sunflower seedlings. Twenty-four-hour-old wheat seedlings were irradiated with doses of x-rays varying from 565 to 13,560 r-units by FRANCIS (**1**). Retardation of fresh weight and dry weight production by the growing parts of the seedlings and retardation of linear growth of the coleoptile, the leaf, the primary root, and the lateral roots was obtained with all doses and time intervals employed. Respiration was likewise depressed with the exception of those seedlings which had received the smallest dose of radiation (565 r-units) and which were tested 5 or 6 hours after irradiation.

The procedure of SHULL and MITCHELL was closely followed in an attempt to obtain further data on the stimulative effects of filtered x-radiation on growth, height, tillering, heading and flowering, green and dry weights, and moisture content of Marquis spring wheat. An attempt to determine the influence of age of seed on response to irradiation was made by the use of two samples of seed; one 57 months old (4 years, 9 months), the other 9 months old. A preliminary trial was made with Fulhio winter wheat.

Methods

Two different samples of Marquis spring wheat seed were used, one 57 months old, the other 9 months old. One lot of older seeds was irradiated on March 14 (experiment 1) and a second lot on March 26 (experiment 2). The lot of seed 9 months old was x-rayed on April 3, 1940. Although treated on the same day the two groups (experiments 3 and 4) were irradiated separately. Fulhio winter wheat was x-rayed and planted on April 16, 1940.

An amount of water equal to 60 per cent. of the air-dry weight of the seed was added to the wheat in Petri dishes and the seed was allowed to germinate for 24 hours at 21° C. At the end of this period the radicles were about 3 mm. long. In selecting grains for irradiation, care was taken to choose those with radicles of uniform length.

Thirty-five seedlings were placed in a Petri dish for irradiation. Seven such groups were x-rayed at a distance of 30 cm. from the target, using a 1-mm. aluminum screen. The x-ray tube was a standard, air-cooled Coolidge Victor tube and was maintained at a potential of 100 pk. kilovolts and a current of 5 milliamperes. At this setting the output of the machine was approximately 38 roentgens per minute. The seven exposures were for 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, and 6.0 minutes which gave doses of 19, 38, 57, 76, 114, 152 and 228 r-units respectively. One group was not irradiated and served as control.

Seven seeds of the irradiated or control grain were planted in each of five 4½-inch pots filled with well mixed garden loam. All plants were given a 16-hour photoperiod, natural daylight being supplemented by 200-watt mazda lamps suspended 24 inches above the tops of the pots. The pots were placed in different positions once a week to obtain identical illumination as far as possible. Measurement of height, from the ground level to the tip of the longest leaf on the leading tiller, was made 14, 21, and 28 days after planting. Six weeks after planting 18 plants were cut at the ground level and green weights, dry weights, and moisture content of the tops determined. Observations regarding heading and flowering were made on the remaining 17 plants. The wheat was assumed to have headed when the head was completely out of the sheath and to have flowered when the anthers were first extruded. At the end of the 56 days these remaining plants were cut at the soil level and green and dry weights determined.

The Fulhio winter wheat was measured at the age of 14 and 28 days. All of the winter wheat plants were harvested when 28 days old and green and dry weights determined. Only the data for the control plants and those receiving 114 r-units were recorded.

Results and discussion

Growth, height, and tillering

The effect of irradiation on the growth and height of plants produced by seeds 57 months old is given in table I. It appears that all of the treatments, 19–228 r-units, resulted in a stimulated growth rate and greater height. While doses of 76–228 r-units resulted in increments in growth and height which were nearly equal, the greatest increase in height (12.8 per cent.) attained at the age of 28 days, was produced by a treatment of 114 r-units. The increased growth in all cases was evidenced by greater

TABLE I

HEIGHTS OF X-RADIATED MARQUIS WHEAT PLANTS FROM 57-MONTH-OLD SEEDS. RESULTS ARE AVERAGES FROM 35 PLANTS

EXPERIMENT	AGE	X-RAY DOSE IN R-UNITS							
		0	19	38	57	76	114	152	228
	days	*in.*	*in.*	*in.*	*in.*	*in.*	*in.*	*in.*	*in.*
1	14	6.71	6.80	7.27	7.44	7.35	6.65	6.64	7.02
	21	9.96	9.97	10.36	10.40	10.91	10.36	9.97	9.37
	28	10.62	10.71	11.30	11.30	11.80	11.50	11.87	10.88
2	14	5.14	6.79	5.68	6.44	5.82	7.72	7.39	7.56
	21	8.86	9.65	8.83	9.83	9.71	10.79	10.17	11.38
	28	10.14	10.89	9.74	11.21	11.41	11.93	11.06	12.49
Average of 1 and 2	14	5.92	6.80	6.47	6.94	6.58	7.18	7.01	7.29
	21	9.41	9.81	0.59	10.11	10.31	10.57	10.07	10.37
	28	10.38	10.80	10.52	11.25	11.60	11.71	11.46	11.68

height, larger stem diameter, and larger leaf area. Only height was measured, however. No difference in color was evident and irradiation had no effect on tillering.

Table II presents the response of plants grown from seeds 9 months old.

TABLE II

HEIGHTS OF X-RADIATED MARQUIS WHEAT PLANTS FROM 9-MONTH-OLD SEEDS. RESULTS ARE AVERAGES FROM 35 PLANTS

EXPERIMENT	AGE	X-RAY DOSE IN R-UNITS							
		0	19	38	57	76	114	152	228
	days	*in.*	*in.*	*in.*	*in.*	*in.*	*in.*	*in.*	*in.*
3	14	9.58	10.82	11.64	11.70	11.24	11.30	10.94	10.72
	21	11.86	13.34	13.51	14.12	13.53	13.20	12.77	13.76
	28	12.95	13.36	15.10	15.61	14.84	14.78	14.05	14.09
4	14	10.36	11.06	11.47	11.46	11.23	11.48	11.24	11.44
	21	13.98	14.50	14.65	14.99	15.48	15.51	15.19	14.75
	28	14.22	15.76	15.99	16.47	16.79	17.06	16.43	15.63
Average of 3 and 4	14	9.87	10.94	11.56	11.58	11.24	11.39	11.09	11.08
	21	12.92	13.92	14.08	14.56	14.56	14.36	13.98	14.26
	28	13.59	14.56	15.55	16.04	15.82	15.92	15.24	14.86

As in the former case all treatments resulted in stimulated growth and height. The height curves, shown in figure 1, reveal that the greatest height was obtained in those plants which had received 57, 76, and 114 r-units. The greatest increment in height (18.0 per cent.) was found in plants which had received 57 r-units of x-radiation when 24 hours old. Tillering was stimulated by treatments of 38–114 r-units; the largest average number of

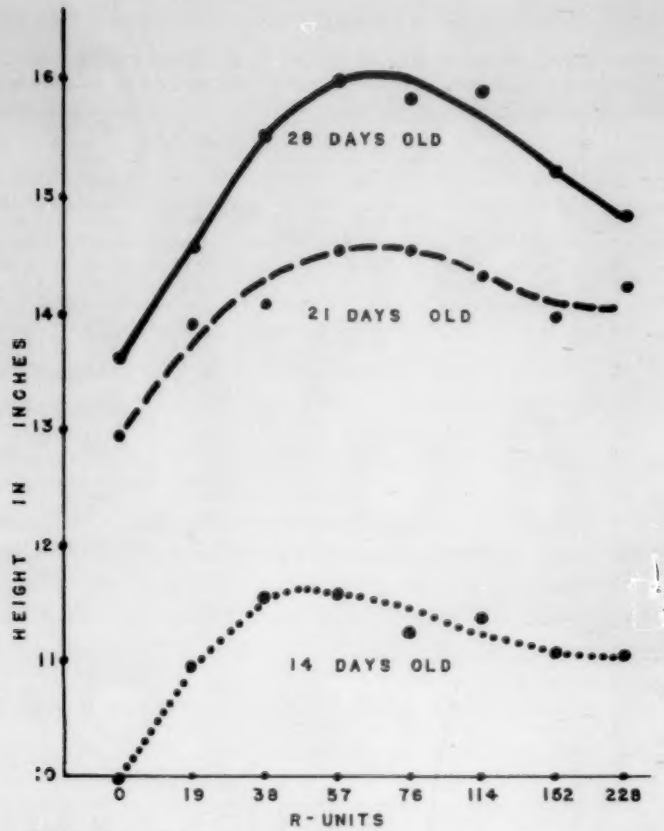

FIG. 1. Heights of Marquis wheat plants when 14, 21, and 28 days old grown from seed 9 months old. The 24-hour-old seedlings were x-radiated with r-unit doses as indicated. Each height is the average for 35 plants (experiments 3 and 4).

culms per plant (1.3) was found on plants irradiated with 76 r-units. Only one head, however, was formed per plant. A decidedly lighter color was observed in all irradiated plants, but the difference was not particularly evident when the plants were 56 days old. As in the experiments with the older seed the stimulus to growth was shown by greater height, stem diameter, and leaf area.

WET AND DRY WEIGHTS; MOISTURE CONTENT

Irradiation of the seedlings from the older seeds resulted generally in increased wet and dry weight; the one exception was an irradiation of 228 r-units which resulted in plants having wet and dry weights equal to those of the controls determined 56 days after planting. Table III gives the

TABLE III

WET AND DRY WEIGHTS AND MOISTURE CONTENT OF X-RADIATED MARQUIS WHEAT PLANTS FROM 57-MONTH-OLD SEEDS. RESULTS FOR PLANTS 42 DAYS OLD ARE THE AVERAGES FROM 18 PLANTS; FOR 56-DAY-OLD PLANTS THE AVERAGES ARE FROM 17 PLANTS

EXPERIMENT	AGE IN DAYS	TEST	X-RAY DOSE IN R-UNITS							
			0	19	38	57	76	114	152	228
1	42	Wet weight*	0.44	0.44	0.71	0.69	0.65	0.65	0.51	0.45
		Dry weight*	0.09	0.09	0.13	0.12	0.14	0.11	0.10	0.11
		Moisture†	80.2	80.6	82.0	82.9	78.6	82.6	80.8	75.5
	56	Wet weight	0.50	0.50	0.71	0.77	0.77	0.61	0.61	0.40
		Dry weight	0.15	0.15	0.21	0.21	0.24	0.17	0.19	0.15
2	42	Wet weight	0.54	0.59	0.65	0.57	0.72	0.56	0.64	0.64
		Dry weight	0.10	0.13	0.12	0.13	0.14	0.13	0.13	0.13
		Moisture	82.1	78.7	81.7	77.7	80.4	77.9	79.3	79.7
	56	Wet weight	0.69	0.66	0.81	0.79	0.97	0.69	0.67	0.79
		Dry weight	0.21	0.19	0.20	0.24	0.26	0.22	0.18	0.21
Average of 1 and 2	42	Wet weight	0.49	0.52	0.68	0.63	0.69	0.61	0.58	0.55
		Dry weight	0.09	0.11	0.12	0.12	0.14	0.12	0.12	0.12
		Moisture	81.2	79.7	81.9	80.3	79.5	80.3	80.1	77.6
	56	Wet weight	0.59	0.58	0.76	0.78	0.87	0.65	0.64	0.59
		Dry weight	0.18	0.17	0.21	0.23	0.25	0.20	0.19	0.18

* Given in grams.
† Percentage of wet weight.

weight and moisture data of experiments 1 and 2. The greatest average wet weight per plant and the greatest dry weight were found in those plants which had received 76 r-units. These weights were greater than those of control plants by 40.8 per cent. and 55.5 per cent., respectively. The various irradiations produced little differences in moisture content of the plants. The average weight values for experiments 1 and 2 are shown graphically in figure 2.

The data of table IV indicate that in the case of fresh seed the higher radiation doses resulted in decreased wet and dry weights compared with control plants. Fifty-seven r-units, however, caused an increase of 67.6 per cent. in wet weight and 23.5 per cent. in dry weight. Moisture content

was not significantly affected by irradiation. The average weight values for experiments 3 and 4 are shown graphically in figure 3.

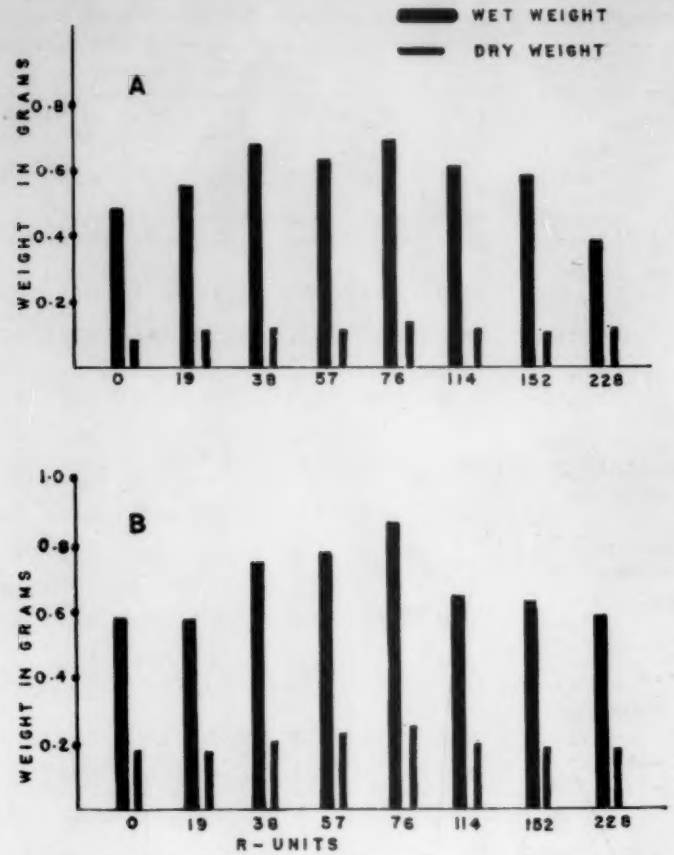

FIG. 2. Wet and dry weights of plants grown from 57-month-old Marquis spring wheat. The 24-hour-old seedlings were given filtered x-ray treatment of the number of r-units shown in the figure. A. Average weights for 18 plants 42 days old. B. Average weights for 17 plants 56 days old.

HEADING AND FLOWERING

An interesting result with respect to heading and flowering is shown by the data of table V. All treatments of the older seed resulted in accelerated heading and flowering, the maximum acceleration being obtained with doses of 76 and 114 r-units. In each case flowering was accelerated by approximately 3 days. The seedlings of the fresh grain, however, were adversely affected by x-radiation, the controls flowering by as much as 6.7 days before the treated plants. That this difference in response may be attributed to

TABLE IV

WET AND DRY WEIGHTS AND MOISTURE CONTENT OF X-RADIATED MARQUIS WHEAT PLANTS FROM 9-MONTH-OLD SEEDS. RESULTS FOR PLANTS 42 DAYS OLD ARE THE AVERAGES FROM 18 PLANTS; FOR PLANTS 56 DAYS OLD, THE AVERAGES ARE FROM 17 PLANTS

EXPERIMENT	AGE IN DAYS	TEST	X-RAY DOSE IN R-UNITS							
			0	19	38	57	76	114	152	228
3	42	Wet weight*	0.75	0.81	1.28	1.49	1.37	1.14	1.14	0.94
		Dry weight*	0.16	0.16	0.20	0.31	0.21	0.16	0.19	0.16
		Moisture†	77.6	80.4	84.2	79.4	84.5	85.9	83.2	83.3
	56	Wet weight	0.91	0.90	1.70	1.69	1.29	1.20	1.18	1.04
		Dry weight	0.31	0.28	0.42	0.43	0.30	0.25	0.29	0.25
4	42	Wet weight	1.00	1.47	1.33	1.41	1.47	1.57	1.09	0.85
		Dry weight	0.20	0.21	0.19	0.21	0.22	0.23	0.16	0.17
		Moisture	79.9	85.4	86.0	84.9	85.4	85.1	84.9	80.0
	56	Wet weight	1.12	1.25	1.55	1.71	1.47	1.58	1.25	0.33
		Dry weight	0.36	0.31	0.36	0.41	0.34	0.39	0.29	0.27
Average of 3 and 4	42	Wet weight	0.88	1.14	1.31	1.45	1.42	1.36	1.12	0.90
		Dry weight	0.18	0.19	0.20	0.26	0.22	0.20	0.18	0.17
		Moisture	78.8	82.9	85.1	82.2	85.0	85.5	84.1	81.7
	56	Wet weight	1.02	1.03	1.63	1.70	1.38	1.39	1.21	0.94
		Dry weight	0.34	0.30	0.39	0.42	0.32	0.32	0.29	0.26

* Given in grams.
† Percentage of wet weight.

the age of the seed is suggested by the fact that growth conditions for all the experiments were as similar as possible with the exception of the slightly greater intensity and duration of daylight in the case of experiments 3 and 4. These experiments were started April 3 as compared with March 14 and March 26 for experiments 1 and 2 respectively. The photoperiod for all experiments was maintained at 16 hours, however.

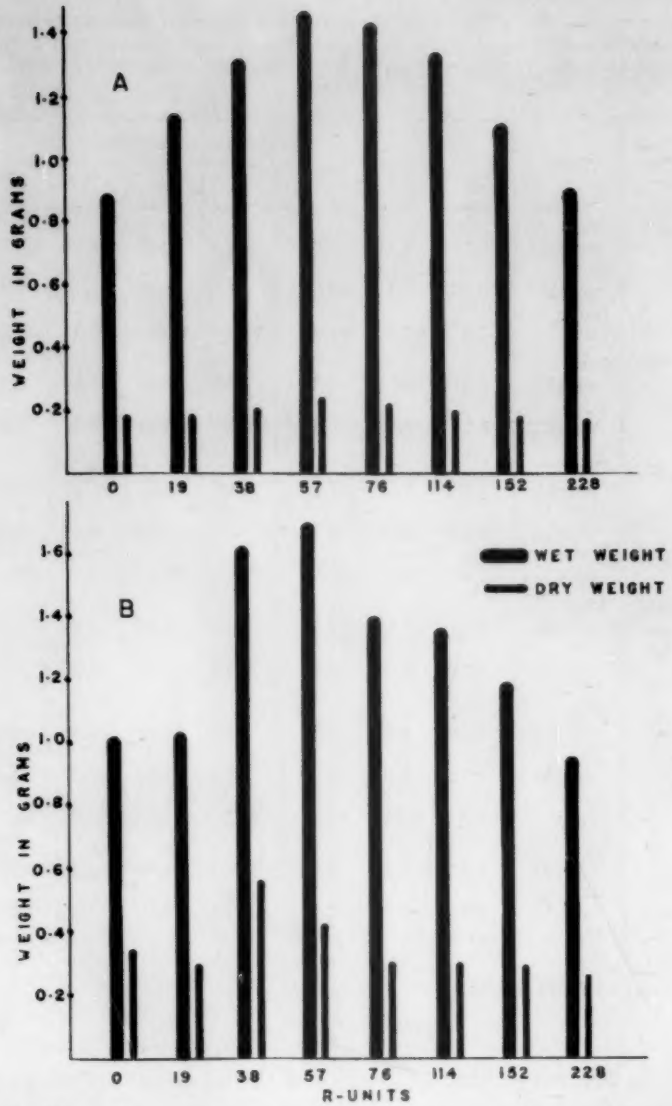

FIG. 3. Wet and dry weights of plants grown from 9-month-old Marquis spring wheat. The 24-hour-old seedlings were given filtered x-ray treatment of the number of r-units shown in the figure. A. Average weights of 18 plants 42 days old. B. Average weights of 17 plants 56 days old.

TABLE V

NUMBER OF DAYS FROM PLANTING TO HEADING AND FLOWERING BY X-RADIATED MARQUIS WHEAT PLANTS FROM SEED 57 MONTHS OLD (EXPERIMENTS 1 AND 2); AND FROM SEED 9 MONTHS OLD (EXPERIMENTS 3 AND 4). RESULTS ARE THE AVERAGES FROM 17 PLANTS

X-RAY DOSAGE	EXP. 1		EXP. 2		AVERAGE OF 1 AND 2		EXP. 3		EXP. 4		AVERAGE OF 3 AND 4	
	HEAD	FLOWER	HEAD	FLOWER	HEAD	FLOWER	HEAD	FLOWER	HEAD	FLOWER	HEAD	FLOWER
r-units	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*	*days*
0	53.1	53.6	49.8	51.2	51.5	52.4	42.0	43.4	42.0	43.7	42.0	43.6
19	51.1	52.0	49.0	49.1	50.1	50.6	42.7	44.2	49.2	50.5	45.9	47.3
38	49.9	51.1	49.7	50.9	49.8	51.0	46.2	47.0	49.6	50.3	47.9	48.6
57	51.4	52.5	45.4	48.0	48.4	50.3	47.0	48.0	49.2	50.3	48.1	49.1
76	47.9	49.1	48.8	49.7	48.4	49.4	48.0	49.7	47.8	48.2	47.9	48.9
114	49.2	50.8	46.7	48.4	48.0	49.6	48.2	49.8	47.1	48.3	47.6	49.0
152	51.4	53.7	47.2	49.0	49.3	51.4	47.0	48.5	51.2	52.1	49.1	50.3
228	50.5	51.5	47.9	49.2	49.2	50.4	45.3	46.8	45.2	46.7	45.3	46.8

Fulhio winter wheat

Irradiation of 24-hour old Fulhio winter wheat seedlings by doses of from 19 to 228 r-units produced stimulation of growth, compared with that of seedlings receiving no treatment. The greatest effects were obtained with a dose of 114 r-units and only these results are recorded in table VI.

TABLE VI

Height, weight, and tiller number of control and x-irradiated Fulhio winter wheat at age 28 days. Results are the averages from 35 plants

	Average height/ plant, age 14 days	Average height/ plant, age 28 days	Average wet weight/ plant, age 28 days	Average dry weight/ plant, age 28 days	Average number of tillers/ plant
	in.	*in.*	*gm.*	*gm.*	
Control	8.08	13.76	0.83	0.12	1.00
Irradiated with 114 r-units	10.72	17.47	1.48	0.21	1.14

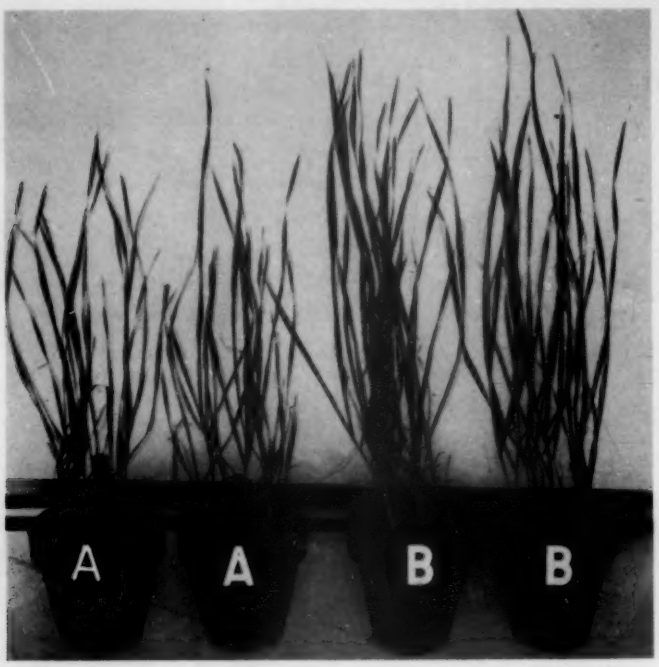

Fig. 4. Irradiated and control Fulhio winter wheat photographed when 28 days old. Those photographed are representative of 35 plants. A. Control. B. Irradiated when 24 hours old with a dose of 114 r-units.

Figure 4 shows the appearance of the wheat when harvested at the age of 28 days.

Summary

1. Twenty-four-hour-old seedlings of Marquis spring wheat grown from seeds 57 months old and 9 months old were irradiated by filtered x-rays of 19 to 228 r-units. Controls were plants receiving no treatment.

2. Irrespective of the age of the seed greatest growth rate and height were produced by 114 r-unit treatments.

3. Greatest fresh and dry weights of plants grown from the older seeds resulted from the use of 76 r-units; of fresh seeds, from 57 r-units.

4. X-radiation of from 76 to 114 r-units accelerated heading and flowering of plants grown from old seed by as much as 3 days, but retardation of heading and flowering occurred when fresh seed was irradiated.

5. Height and weight of Fulhio winter wheat seedlings were increased considerably by all doses of x-rays used, the maxima occurring when the dosage was 114 r-units.

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PLANT CULTURE EQUIPMENT

FRANK M. EATON

(WITH FOUR FIGURES)

Introduction

A detailed description is presented in this paper of small sand and water culture equipment. A number of large out-of-doors sand cultures both for tree and annual crops have also been constructed at the U. S. Regional Salinity Laboratory, Riverside, California. In their general features these latter cultures are like those described in an earlier publication (**3**). The more significant modifications are discussed.

The same principle of operation is used in all of the sand culture equipment. Briefly stated, each sand culture consists of a sand bed and a solution reservoir. Nutrient solution is pumped from the reservoir onto the surface of the sand at hourly or other selected intervals. The solution applied to the surface of the sand displaces to the reservoir a large part of the solution remaining in the sand from the previous flushing. Drainage to the reservoirs is provided by the use of a permeable aluminum silicate material manufactured in the form of bricks for the insulation of furnaces. These bricks (or discs) confine root growth to the sand and thus prevent the stoppage of drains. Inasmuch as the intervals of application of solution to the sand are controlled by time clocks, a minimum of manual attention is required.

Small sand cultures

The stoneware cultures, figures 1 to 3, were designed for nutritional studies in the greenhouses where the effects of a number of treatments are compared in replicated cultures. While the equipment shown was intended primarily for small plants, its usefulness for large plants is indicated by the tomatoes in figure 2. For use with large plants advantages of larger reservoirs are indicated, since the frequency with which new solutions are substituted for old ones, or the frequency with which determinations and additions of nutrient ions are made, can thereby be reduced. The pottery company that has cooperated in the manufacture of the stoneware has advised that they can build 35- or 40-liter stoneware reservoirs with bell-shaped tops that would accommodate the present sand trays and other fittings.

CHAPMAN'S and LIEBIG'S idea (**1**) of using air-lift pumps has been adopted in these cultures. These investigators made use of large earthenware tile reservoirs set in greenhouse floors.

The sand cultures shown in figure 3 are mounted on a rotating table to produce uniform conditions in an unevenly lighted greenhouse. Com-

pressed air is brought into the center of the revolving table through a mercury sealed connection. The revolving portion of the table top rests on the wheel assembly of a heavy truck trailer. The axle of this assembly is set in a concrete-filled excavation beneath the greenhouse floor.

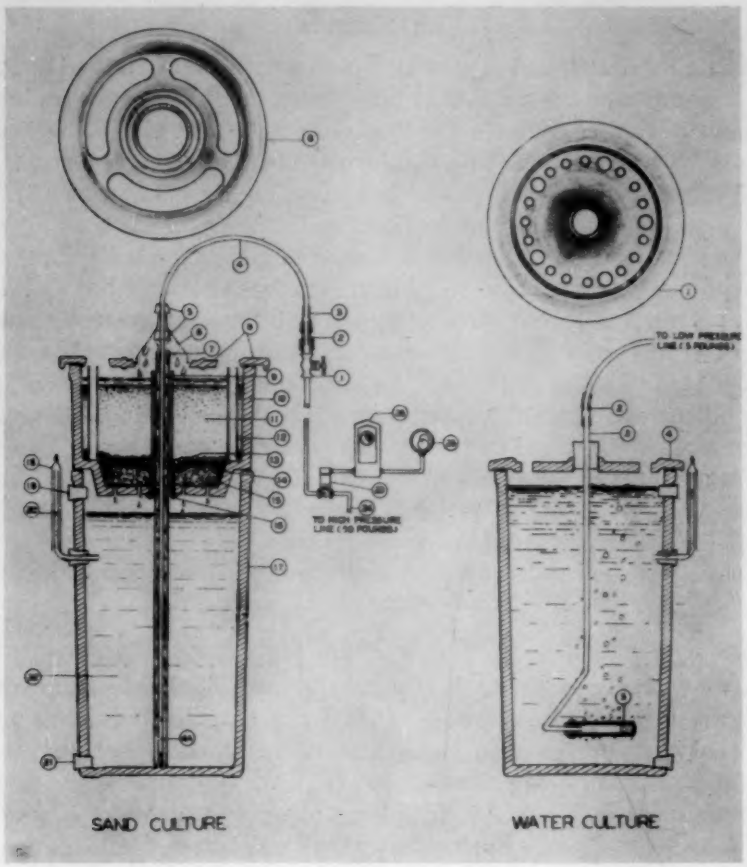

FIG. 1. Cross section view of sand culture (left) and water culture (right).

The following paragraphs, numbered to correspond to the designations of the parts in figure 1, left, describe the character of the various pieces of the sand culture equipment:

1. *Air valve.*—This is a ¼-inch needle valve of the less expensive type. It is used for adjusting the rate of flow of air from the outlet at 4a. The valve has male threads at both ends (fig. 2, left).

2. *Hose connector.*—This ¼-inch acetylene-welder type of connection has

FIG. 2. Sand cultures on greenhouse benches.

FIG. 3. Sand cultures mounted on a rotating table in unevenly lighted greenhouse.

a hexagonal nut which permits ready disconnection of the air line when the cultures are to be dissembled.

3. *Koroseal tubing.*—Tubing of this type[1] (suggested by L. A. RICHARDS) stands up under greenhouse light conditions much better than rubber tubing. It is heated in a water bath before being set in place.

4. *Air-discharge tubes.*—Soft aluminum tubing with ¼-inch outside diameter. A 1/32-inch hole is bored through one wall at 4a to emit air. The lower end is closed by clamping it in a machine vise.

5. *Funnel supports.*—These are the two halves of a no. 2 rubber stopper.

6. *Aluminum funnel.*—Outside diameter of base, 2¾ inches.

7. *Solution delivery tube.*—Hard thin-walled aluminum tubing 22¾ inches long with ¾-inch outside diameter. V-notches ¾-inch deep are cut on opposite sides of the lower end to provide inlets for the solution. The delivery rate of solution by this tubing with an inside diameter of 17 mm. in single tests was 2.6 liters per minute compared with a rate of 1.3 liters per minute from a tube with 13-mm. inside diameter. Corrosion of this tubing can be delayed by dipping it in Bakelite varnish.[2]

8. *Glazed stoneware covers.*—These are used to suppress growth of algae and check surface evaporation. Slips of sheet aluminum or cardboard with U-shaped notches for the plant stems are laid over the openings in the cover after the plants have been thinned to final number.

9. *Glass sleeve and overflow.*—Pyrex glass tubing with inside diameter of 22 mm. and outside 30 mm., length 17 cm. To seal in place, the glass tube is extended through the circular opening in porous disc, 15, and bottom of the tray, 10, letting the lower end of the tube rest on the work bench. After placing a centimeter depth of coarse sand in the bottom of the V-shaped opening around the porous disc, hot G–K compound, 14, is poured in with a spoon until the groove is filled. A thin rim of G–K compound is then built up on the disc around tube. When this material has hardened, the tray is inverted and hot red sealing wax is added to fill up the space between the tube and rim of the opening in the bottom of the tray. The top of the overflow tube should now be about 1.5 cm. below the top of the tray.

10. *Sand tray.*—This piece of stoneware has a volume of about 6.5 liters.

11. *Quartz sand and magnetite.*—Five kilograms of sand is mixed with 25 grams of powdered magnetite, $FeO \cdot Fe_2O_3$. The layer of gravel in the bottom of the tray should be wetted before adding the sand. A sand, of which 50 per cent. is retained by a 60-mesh screen after passing a 40-mesh

[1] Supplied by B. F. Goodrich Company, size 3/16-inch inside diameter, with 1/8-inch wall thickness.

[2] A hard rubber tubing supplied by Kirkhill Rubber Company, Los Angeles, California, with 22/32-inch inside diameter and 25/32-inch outside diameter, also appears to be satisfactory in preliminary trials and it is somewhat less expensive.

screen, has been found to be satisfactory.[3] In solutions at pH 6, the magnetite has supplied ample iron to all plants tested (**1, 2, 3**).

12. *Air vents.*—Without these glass tubes which extend into the gravel, air is locked in the sand when the trays are flushed, thus preventing a free, uniform, downward movement of solution.

13. *Quartz gravel.*—One kilogram of gravel of a size which passes a 4-mesh screen and is held on an 8-mesh screen is suitable. The sand does not work down into the gravel if the latter is wetted before filling.

14. *Asphalt compound.*—This product, which is sold under the trade name G–K sewer-joint compound, has a rather low melting point, but it maintains a good union with the glazed walls of the sand tray when it cools.[4]

15. *Porous disc.*—These are purchased in $6\frac{1}{4}$-inch squares 1 inch thick. A 30-mm. hole is first bored through the center on a press drill with a steel tube. From 10 to 14 of them are then strung on a 30-mm. rod, clamped into a lathe, and then turned down to a diameter of $6\frac{1}{8}$ inches. The material works easily. The permeability of the discs is about 600 ml. per minute when the trays are filled with sand and the solution is at the height of the overflow.

16. *Red sealing wax.*—This is used to seal the overflow tubes, 9, into the bottoms of the sand trays. Five grams of finely powdered sealing wax added to a liter of culture solution has been found to be somewhat toxic to tomato plants in water cultures. The small solid piece used in this seal has shown no ill effects.

17. *Reservoir.*—The jars shown have a capacity of 14.5 liters. They have three outlets, each of which takes a no. 2 rubber stopper. The opening at 19 is set 4 inches to the right of those at 20 and 21.

18. *Cap for water gauge.*—This is a piece of $\frac{1}{2}$-inch aluminum tubing with one end closed. Its use prevents the growth of algae in the gauge.

19. *Water-addition opening.*—In replenishing solutions, this stopper is removed and the point of a self-closing radiator faucet inserted.

20. *Water gauge.*—This is a 5- or 6-mm. glass tube bent in the shape shown and painted black below cap.

21. *Outlet for emptying reservoirs.*

22. *Culture solution.*—Salts for 13 liters are used in making up 11 liters of solution. Two liters of water are then added to the surface of the sand and when draining has stopped, a wax mark is made on the water level gauge to indicate the initial volume. The five kilograms of sand retain between 1.6 and 1.8 liters of solution against gravity.

[3] This sand is supplied by the Industrial Sands Division, Corona, California.

[4] Atlas Mineral Products Company, The Deming Company, 4227 Whiteside Avenue, Los Angeles, California.

23. *Magnetic air valve.*[5]

24. *Air pressure line.*—Air pressures up to about 50 pounds are satisfactory. Where a large number of cultures are operated from a single air line, the latter pressure beyond the pressure regulator is desirable. A discharge rate of 0.7 cu. ft. of air per minute per culture gives a satisfactory solution delivery rate.

25. *Time switch.*—The General Electric TSA–14 time switch can be used alone without time-switch 26 if the cultures are flushed each hour throughout the 24 hour period. This timer makes a complete cycle every 60 minutes. The duration of the "on" period can be set for any fraction of the 60-minute period. Three minutes are ample for the complete replacement of the solution held by the sand, providing air pressures are maintained.

26. *Time switch.*[6]—This switch is connected in series with switch 25 in such a way that the circuit to the magnetic valve is completed only during an "on" period of both timers. The "on" and "off" pins on the 24-hour dial of the Sangamo timer cannot be inserted nearer together than 15 minutes. This switch is used to limit the flushing of the sand trays to selected hours.

Water cultures

In using the sand-culture solution reservoirs as water cultures, the air hose leading from each greenhouse bench is disconnected from the non-continuous high-pressure air line and attached to a continuous low pressure outlet. Carbon-tube aerators are employed following a suggestion originating with P. R. Stout and D. I. Arnon of the University of California.

1 and 4. *Glazed stoneware cover.*—Two sizes of tapered holes are provided in this cover for mounting seedlings of various sized plants. The cotton mountings for seedlings stay in place better if the holes are not glazed.

2. *Rubber or Koroseal connection.*

3. *Glass or ¼-inch aluminum tubing.*

5. *Aerator.*—Plain carbon pipe,[7] diameter inside ½-inch, outside ¾-inch. Five pounds of air pressure can be used without blowing the cork stoppers if they are inserted after having been dipped in hot G–K Compound.

Stoneware

The stoneware described is manufactured[8] in accordance with specifications. Plain solid covers to fit trays and reservoirs of this size are available at nominal cost.

[5] Made by Magnetic Controls.

[6] Sangamo Type TC–11 (sold by Graybar Electrical Co.).

[7] Supplied by National Carbon Company, Inc.

[8] By the Pacific Clay Products, 306 West Avenue 26, Los Angeles, California. Reservoirs (U. S. 3), $1.00; sand trays (U. S. 4), 60 cents; water-culture covers (U. S. 5) 50 cents; and sand-tray covers (U. S. 6), 60 cents.

The squares of insulating brick were cut to order (1 inch by 6¼ inches).[9] There are a number of insulating bricks of similar composition and volume weight on the market, but the Vitrefrax has been the only one so far tested that had a high permeability.

The other pieces of the equipment are all standard products and the prices would accordingly vary with location and purchasing conditions.

Large out-of-doors sand cultures for annual and tree crops

The new sand cultures for annual crops grown to maturity, figure 4, have dimensions similar to the one previously described (3, fig. 2) except that the

FIG. 4. Out-of-doors sand cultures for annual crops.

depth of the reservoir has been increased from 18 to 29 inches, giving a capacity of 2,400 liters. Asphalt painted transite in pieces ¼-inch thick are used in place of tin to suppress the growth of algae. The motor pump is placed in a housing at the end of the bed below the upper level of the solution. Aluminum silicate brick are used for drainage in place of gravel. The ends of 39 standard size brick ($2 \times 4.5 \times 9$ inches) are bevelled so that they rest on the correspondingly bevelled edges of the concrete cross tile. The walls of these unit type beds are 6 inches thick, thus permitting the use of a recess rather than angle iron to support the transite.

[9] Furnished by the Vitrefrax Corporation, 5050 Pacific Blvd., Los Angeles, California, in lots of 100 at 35 cents each.

The 18 deep sand beds for tree crops at the U. S. Regional Salinity Laboratory, Riverside, California, have inside dimensions of $5 \times 10 \times 6$ feet deep. They are spaced 10×18 feet center to center. Whole permeable brick are laid above drainage ways cast in the concrete floor. A number of tubes for the escape of air during flushing are extended into the gravel laid above the permeable brick. The inside dimensions of the reservoirs are $7 \times 4 \times 6$ feet deep.

A high silica cement-lined pipe sold under the trade name "Duroline" is used for all plumbing with promising results from the standpoint of corrosion.

The concrete walls of these cultures are made nearly impermeable and chemically inert by a two-coat sprayed-on dressing of emulsified asphalt.[10]

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[10] The material used is sold under the name "Static Protective Coating" by The Flintkote Company.

ANTAGONISTIC ACTION OF CHLORIDES ON THE TOXICITY OF IODIDES TO CORN[1]

J. C. Lewis and W. L. Powers

During recent investigations of the relation of iodine to plant nutrition, we found iodides to exert toxic effects on corn at very much lower concentrations than had previously been reported by Mazé (**5**). Mazé's nutrient solutions contained 22 p.p.m. of added chlorides. Our solutions contained only such concentrations of chlorides as were carried as impurities by the salts and water used in their preparation. We therefore conducted experiments to determine whether chlorides antagonize the toxic effect of iodides on corn.

Methods

Corn seeds were germinated in the basal nutrient solution described below. When 3 to 4 inches high, uniform seedlings were transferred to 4-gallon stoneware jars containing the basal nutrient solution. Iodide and chloride additions were made 2 to 3 days later.

The basal nutrient solution used had the following composition: calcium nitrate, 0.007 M; magnesium sulphate, 0.005 M; monopotassium phosphate, 0.005 M; dipotassium phosphate, 0.0015 M; boron as boric acid, 0.5 p.p.m.; manganese as sulphate, 0.5 p.p.m.; zinc as sulphate, 0.05 p.p.m.; copper as sulphate, 0.02 p.p.m.; and molybdenum as ammonium molybdate, 0.05 p.p.m.

Tap water was used in the preparation of this solution. Ferric tartrate was added twice weekly at the rate of 1 ml. of a 0.5 per cent. solution per liter of nutrient solution. In one experiment, humate iron (**3**) equivalent to 2 p.p.m. of iron was used in place of ferric tartrate. The initial pH of the basal nutrient solution was approximately 5.8. The pH of the culture solutions was adjusted every 2 weeks, or as needed, while the solutions were renewed at 30-day intervals.

Iodine was added as potassium iodide. Chlorides were added as a mixture of the calcium, magnesium, and potassium salts, in the same cation proportions as these cations appeared in the basal formula.

The methods used for iodine analyses varied with the anticipated iodine content. High-iodine samples were fused with potassium hydroxide. Low-iodine samples were burned in the von Kolnitz-Remington (**7**) oxygen torch. The iodine was then recovered by the distillation method of Bratton and McClendon (**2**), followed by bromine oxidation and thiosulphate titration.

[1] Published as Technical Paper no. 362 with the approval of the Director of the Oregon Agricultural Experiment Station as a contribution of the Soils Department.

Chlorine was determined by the A.O.A.C. (1) gravimetric method after a nitrous acid treatment (6) designed to remove iodine.

Results

The results of our first experiments to determine the toxic levels of iodides for corn are given in table I. The growth period was 60 days, by

TABLE I

EFFECT OF IODIDES ON GROWTH AND COMPOSITION

IODIDE ADDED TO SOLUTIONS	MEAN DRY WEIGHT OF TOPS	IODINE CONTENT OF TOPS	TOTAL IODINE IN TOPS	CHLORINE CONTENT OF TOPS	TOTAL CHLORINE IN TOPS
p.p.m.	*gm.*	*p.p.m.*	γ*	*p.p.m.*	*mg.*
0	17.2 ± 2.0†	0.05	0.9	.....	.....
0.1	14.0 ± 1.8	8.6	120	1510	21.2
0.5	11.8 ± 1.8	58	680	1600	18.9
1	9.9 ± 1.5	88	870	1850	18.3
5	2.2 ± 0.4	439	950	7820	17.3

* The microgram (γ) is 0.001 mg.
† The standard error of the mean is used throughout this paper.

which time the corn had immature ears. Duplicate jars with 3 plants per jar were used for each treatment, except that in which 5 p.p.m. of iodine was added for which only 1 jar was used.

The data show growth repression even at 0.1 p.p.m. of added iodine, while 5 p.p.m. is extremely toxic. Chlorosis is pronounced in the presence of 5 p.p.m. of iodine. The iodine content of the tops is approximately proportional to the iodine concentration of the nutrient solutions. The chlorine content appears to be related to the iodine concentration of the nutrient solutions.

The next two experiments were conducted to determine the effect of 20 p.p.m. of added chlorides on the toxic action of 2 p.p.m. of iodine. In the first of these experiments, humate iron was used as a source of this element. These plants became uniformly chlorotic, and growth was greatly repressed. Local additions of iron salts on the leaves produced green spots, showing that the symptoms were caused by iron deficiency. These plants are referred to hereafter as "iron-deficient."

In the second experiment ferric tartrate was used as a source of iron. These plants, which did not suffer from iron deficiency, are referred to hereafter as "normal." After 30 days the plants in each jar were weighed and those 4 nearest the mean weight were returned to the culture solutions. After a total of 46 days these plants had reached the tasseling stage and were then harvested. Triplicate jars were used for each treatment. Eight seedlings per jar were used.

The results, summarized in table II, indicate that 2 p.p.m. of iodine reduced the yields by approximately 40 to 60 per cent. About one-half of this injury was preventable by the addition of 20 p.p.m. of chlorides.

TABLE II

EFFECT OF CHLORIDES ON THE TOXICITY OF IODIDES

TREATMENT ADDED TO SOLUTION		IRON-DEFICIENT CORN	NORMAL CORN	
		GREEN WEIGHTS AFTER 27 DAYS	GREEN WEIGHTS AFTER 30 DAYS	GREEN WEIGHTS AFTER 46 DAYS
		gm.	*gm.*	*gm.*
Chloride	0 p.p.m.	6.35 ± 0.37	27.0 ± 1.0	120 ± 8
Iodide	0 p.p.m.			
Chloride	20 p.p.m.	7.57 ± 0.34	28.5 ± 1.1	108 ± 5
Iodide	0 p.p.m.			
Chloride	0 p.p.m.	3.61 ± 0.17	16.3 ± 1.3	44 ± 5
Iodide	2 p.p.m.			
Chloride	20 p.p.m.	6.07 ± 0.29	21.7 ± 1.0	80 ± 9
Iodide	2 p.p.m.			

Chlorine and iodine analyses of certain of the plant materials were made to see if the chlorides act by repressing the absorption of iodine. These data, presented in table III, indicate that the iodine content has not been especially affected by the chloride concentration of the solutions. The chloride content, in most cases, has been markedly increased by the presence of 2 p.p.m. of iodine in the cultural solutions.

TABLE III

EFFECTS OF IODIDE AND CHLORIDE TREATMENTS ON COMPOSITION OF CORN PLANTS

TREATMENT ADDED TO SOLUTIONS		30-DAY-OLD PLANTS		46-DAY-OLD PLANTS					
		TOPS	ROOTS	LEAVES		STEMS		ROOTS	
		CHLORINE	CHLORINE	CHLORINE	IODINE	CHLORINE	IODINE	CHLORINE	IODINE
		p.p.m.	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*	*p.p.m.*
Chloride	0 p.p.m.	2940	2070	1410	0.46	1320	0.14	840	0.42
Iodide	0 p.p.m.								
Chloride	20 p.p.m.	5560	2980	5520	0.33	4750	0.14	2050	0.51
Iodide	0 p.p.m.								
Chloride	0 p.p.m.	4540	2650	2860	236	2460	166	1440	138
Iodide	2 p.p.m.								
Chloride	20 p.p.m.	7560	3490	5980	240	5630	156	1690	136
Iodide	2 p.p.m.								

Tap water was used in these experiments since previous work had shown that nutrient solutions prepared from tap water and C. P. chemicals had iodine contents of the order of 0.001 p.p.m. Corvallis city tap water has had an average iodine content for the past several years of 0.00035 p.p.m.

After analyses were made of the plants grown in solutions to which chlorides had not been specifically added, it became apparent that the basal nutrient solution contained very appreciable amounts of chlorides. Subsequent analyses indicated a chloride content of about 3 p.p.m. in the tap water, while the chlorine contributions from the nutrient salts and the seeds were negligible.

Discussion

Under the experimental conditions the extent to which chlorides antidote the toxic action of an iodide concentration that gave a 50 per cent. reduction in yield was quite marked and fairly uniform. The magnitude of this degree of toxicity is well suited to studies of this type. The reduction in yield is marked enough to give clear-cut results without serious interference from secondary factors such as chlorosis. No work was done to determine protective Cl/I concentration ratios such as those worked out for other chemically-related elements by HURD-KARRER (**4**).

Since merely raising the chloride concentration in the nutrient solution from approximately 3 p.p.m. to 23 p.p.m. gave a substantial antidoting effect, one is led to speculate that the toxicity of a given level of iodides would have been greater had chlorides been more rigorously excluded from the culture solutions. Likewise, the response of iodide toxicity to added chlorides may depend on the amount of chlorides already present in the solutions and may be greater at lower basal chloride concentrations.

In table III it may be seen that the chlorine contents of the 30-day-old normal corn are considerable higher than those of the 46-day-old normal corn. Moreover, iodide treatment is almost as effective as chloride treatment in raising the chlorine content. The possibility of an artifact wherein iodides would be mistaken for chlorides in the analyses was eliminated by removal of iodine by nitrous acid (**6**) treatment. This separation was found by actual test to be complete under our conditions.

The effect of iodine in increasing the chlorine content may be most readily explained by assuming that the primary effect of the iodine is in inhibiting growth, while it has little effect on the absorption of chlorides. In this event the total chlorine absorbed per plant should be relatively constant. That this is the case may be seen in table I. Similar calculations for the other experiments are in agreement with these data.

In general, the iodine content of the plants is approximately proportional to the iodide concentration in the solutions. The absolute amount of iodine in the plants is therefore determined in part by the effect of iodine on

growth, as well as by the iodine content of the plants. The chloride concentration in the solutions apparently does not affect the iodine content of the plant tissues.

Since chlorides do not appear to act by repressing iodine absorption, they may act by replacing iodine in certain physiological reactions within the plant. This general reaction was proposed by HURD-KARRER (**4**) in her "mass-action antagonism" hypothesis. In this case, the increased chlorine content of the plant may be a protective response of the plant to iodide toxicity, the plant maintaining a similar chloride absorption rate per plant in spite of the reduced growth rate.

The apparent lack of relation between iron deficiency and the chloride-iodide antagonism is noteworthy. Extreme iodide toxicity (5 p.p.m. in table I) is superficially very similar to severe iron-deficiency chlorosis. Corn grown in solution cultures is very susceptible to iron deficiency. This may have contributed to some extent to the variability of the individual plant weights obtained in these experiments.

It is of interest to note that marine plants, and especially seaweed, normally have iodine contents that may be acquired by land plants only in the presence of highly toxic iodine concentrations. In the case of marine plants, the plentiful supply of chlorides in the environment may be of prime importance in permitting the accumulation of iodine without toxic effects.

Of more immediate importance is the fact that the discovery of the iodide-chloride antagonism permits a partial explanation of certain discordant results in the literature dealing with the effects of iodine on plant growth. The presence of an adequate concentration of chlorides, by reducing the toxic action of iodides, may profoundly influence the outcome of such experiments.

Summary

1. The addition of 20 p.p.m. of chlorides partially prevented the toxic action of 2 p.p.m. of iodides on corn in solution cultures.

2. The iodine content of the plant material was not affected by the chloride concentration in the solutions, but the chlorine content was increased markedly in the presence of 2 p.p.m. of iodides.

3. The iodide-chloride antagonism appeared to be independent of iron deficiency.

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SOME COMMENTS ON THE MECHANISM OF PHLOEM TRANSPORT

T. G. Mason and E. Phillis

In a recent paper, Crafts (**6**) points out that "Present theories fall into two categories: (1) movement of solute molecules taking place in, through, or upon the surface of sieve-tube protoplasm, and which results from protoplasmic activity; (2) mass flow of solution through sieve-tubes or phloem, related, at least indirectly, to activity of photosynthetic tissues, and not dependent upon the activity of the sieve-tube protoplasm." His paper presents his objections to the first mechanism and the case for mass flow.

"The pressure-flow theory," he now says, "pictures acceleration in the sieve-tube as resulting from a hydrostatic pressure, developed osmotically and exerted impartially upon solute and solvent." "Conduction," he thinks, "is permitted by a high permeability of the sieve-tube cytoplasm resulting from a decline in its activity state."

The prototype of pressure-flow theories is that advanced by Münch (**16**). One prerequisite of this theory is open sieve-pores,[1] and provided that those are present, Mason, Maskell, and Phillis (**12**) concluded that the observed gradients of osmotic pressure were adequate to produce the required rate of flow through the sieve-tubes. But all recent investigators are unanimous in concluding that the sieve-pores are not open. Crafts (**3**) stressed this fact and his recognition of it led him to suggest that the pressure stream travelled longitudinally through the walls. The fallacy in his calculation of wall resistance was exposed by Steward and Priestley (**22**).

Crafts (**6**) has since modified his position by postulating movement along the lumen of the sieve-tube and through the transverse walls separating sieve-tubes. The protoplasm of the functional sieve-tube, he believes, has lost the property of semi-permeability. Presumably there would also be some longitudinal movement through the walls of the phloem parenchyma and the sieve-tubes. His belief that the sieve-tubes are freely permeable is based on his inability to plasmolyze these elements. He seems to think that the sieve-tubes become senescent before the other phloem elements. In a previous paper (**14**), we have pointed out that sieve-tubes may be plasmolyzed provided they are not injured. Curtis and Asai (**7**) have very recently confirmed our observations. There would thus appear to be no experimental grounds for Crafts's belief that the sieve-tubes are freely per-

[1] The vacuoles of adjacent sieve-tubes were assumed to be continuous through the sieve-pores.

meable.[2] Apparently he has not realized that the sieve-pores allow pressure to be transmitted from one element to another; thus sieve-tubes might be injured[3] at some distance from a cut. As to the senility and low activity of the sieve-tube, SMALL (**21**) has recently demonstrated that protoplasmic streaming occurs in these elements. One wonders whether the sieve-tubes are always as short-lived as CRAFTS believes. In palms, for instance, it would appear that they often live for a great many years.

CURTIS and ASAI (**7**) have also pointed out that if the sieve-tubes are permeable to their contents there would be lateral leakage since there is nothing equivalent to the Casparian strip in the stem. They found that "the phloem exudates from Fraxinus and that from Cucurbita will strongly plasmolyze their respective phloem parenchyma cells" and point out that this "demonstrates that the sieve-tube membranes cannot be completely permeable to their contents." A further point is that hydrostatic pressure could not be generated in the fine veins of the leaf (**17**) if the transition cells were freely permeable.

Even if the sieve-tube could not be plasmolyzed, it would only indicate (apart from the possibility that it had been killed) that its protoplasm was equally permeable to water and solutes. It would not follow that its permeability to water had been affected. CRAFTS's modification of the MÜNCH theory involves movement through highly permeable protoplasm instead of movement through an open channel. The observed pressure gradients were only just sufficient to account for movement through open sieve-pores and not through sieve-pores filled with a viscous colloid, which is what CRAFTS's suggestion amounts to. Actually the capacity of the phloem to conduct water appears to be extremely limited. Thus, when the wood is removed from the stem of a cotton plant between the foliage region and the root, the leaves rapidly wilt. Furthermore, when all the leaves are removed, the unlignified upper part of the stem fails to remain turgid. The observations of DÖPP (**9**), who has shown that the growth of young fruits of apple is completely inhibited if the wood leading to them is broken, point in the same direction.

We now enter territory that is still somewhat debatable. The pressure-flow theory requires a hydrostatic pressure gradient in the conducting tracts. It has been generally assumed that this gradient is caused by differences in osmotic pressure. Osmotic pressure gradients of 2 to 9 atmospheres per meter in the sap exuded from sieve-tubes have been demonstrated by DIXON (**8**). BENNET-CLARK, GREENWOOD and BARKER (**1**) have recently

[2] It is of interest to note that the cells of the bast-glomeruli of the yam (**10**) can be readily plasmolyzed.

[3] In a recent paper (**19**) we have considered some of the factors responsible for the production of sap from protoplasm as a result of pressure injury.

suggested that the turgor pressure of the cell is only partly due to the osmotic pressure of the vacuole. They postulate in addition a secretion pressure driving water into the vacuole. Our own observations on the osmotic pressure of the vacuole (**15**) have led us to suggest that the contribution made by the osmotic pressure is very small compared with that of the secretion pressure. Our suggestion has since received confirmation from the very recent work of BENNET-CLARK and BEXON (**2**). It would thus appear that the whole question of the existence of hydrostatic pressure gradients in the sieve-tube system is in doubt.

In commenting on what he terms "the inadequacy of mass-tissue analysis for studies on translocation," CRAFTS (**6**) says "mass analysis gives predominantly a picture of vacuolar concentrations and indicates simply the amount of solutes maintained in the vacuoles." We have recently shown (**15**) that the concentration of solutes in the vacuole is only a small fraction of that in the protoplasm. Our results have since been confirmed by BENNET-CLARK and BEXON (**2**). We were also able to show that the protoplasm normally occupies the bulk of the cell. Mass analysis therefore gives predominantly a picture of the concentration in the protoplasm and is accordingly justified in studies on translocation. Thus, in the leaf, over 90 per cent. of the sugar may be in solution in the protoplasm.

CRAFTS remarks that "continued experiments on chilling and coating petioles have failed to prove that sieve-tube protoplasm plays an essential rôle in longitudinal transport." But we have shown (**13**) that withholding the supply of oxygen to the stem results in a stoppage of transport only as long as oxygen is withheld, and that reducing the supply of oxygen to defoliated fruiting branches retards the rate of carbohydrate transport to the boll. CRAFTS apparently believes that these results may have been caused by altered permeability. It will be evident that this explanation is in direct conflict with his statement that such "experiments have failed to prove that sieve-tube protoplasm plays an essential rôle in longitudinal transport."

CRAFTS (**5**) is now sensible that mere exudation of sap is not evidence of a directed mass flow of solution through the sieve-tube system (**22**). He has accordingly attempted to show that the exudate is not of local origin. He claims that on cutting away 36 cm. of stem from a squash plant the volume of the exudate was equal to the volume of phloem in 173 cm. of stem. The stem was cut repeatedly in thin slices. He remarks that "during the collection several internodes were removed." He says nothing about the leaves and petioles on the nodes. Until it is clear how much sap was contributed from this source, his claim that the exudate is not of local origin must remain in suspense. His belief that the exudate depends on the activity of the photosynthetic tissues would appear to be without foundation, for

we have found that active exudation proceeds from pumpkin stems a number of days after the leaves had been removed, while he himself (**4**) found that "the exudation from the cut peduncle of a fruit stored for six weeks was normal." As far as we are aware it has not yet been demonstrated that the direction and rate of transport are in any way related to the direction and rate of exudation (**11**).

The calculations of MASON and MASKELL (**11**) led them to conclude that the diffusion constant of sugar in the phloem is almost forty thousand times as great as the diffusion constant in a 2 per cent. solution of sucrose in water. CRAFTS (**6**) points out that these rates are based on the whole area of the phloem, including sieve-tubes, companion cells, parenchyma, and the cell walls of these cells and are therefore much too small. He concludes that sugars must move in the sieve-tubes at rates at least 200 times those originally proposed by MASON and MASKELL. While he may be correct in concluding that MASON and MASKELL's calculations are too low, it is very doubtful if they ought to be raised as much as he suggests. He claims to have measured, for instance, the thickness of the cytoplasmic layer of the sieve-tube, even though SINNOTT and TROMBETTA (**20**) concluded that "the problem of determining cytoplasmic volume in plant cells seems almost a hopeless one." We have referred in a recent paper (**15**) to the difficulties involved in measuring protoplasmic volumes. Without going through the whole of his calculations we should refer to his failure to make any correction for the collapse of the sieve-tube when sections are cut. On the other hand, the figures of MASON and MASKELL may be too high for two reasons. In the first place, the sugar gradients in the sieve-tubes may be steeper than in the phloem parenchyma. Secondly, SMALL (**21**) has demonstrated protoplasmic streaming in mature sieve-tubes. While streaming alone could not account for the acceleration of diffusion at the rates calculated by MASON and MASKELL, the existence of streaming would greatly reduce the diffusion constants required to move carbohydrates at the observed rates. The point to stress in connection with the calculations of MASON and MASKELL of the diffusion constant for sugar in the sieve-tube is that it is immeasurably greater than the diffusion constant of sugar in water.

To those who believe that protoplasm has a gel structure consisting of a predominantly protein reticulum and an aqueous continuous phase, such diffusion rates will be incomprehensible. Protoplasm, however, has almost certainly not a typical gel structure. However this may be, it has structure, and structure that exists only as long as metabolic energy is supplied. Energy is utilized in maintaining structure and, in virtue of this structure, protoplasm has properties quite unlike those of water. Moreover, the amount of energy required to maintain this structure would bear no relation to the amount of energy that would be required to accelerate diffusion

in water. The hypothesis of *activated diffusion*, in short, regards protoplasm as an *activated* liquid with diffusion constants and solubilities (**18**) quite different from those of water. Until the physicist knows more concerning the nature of liquids (*e.g.*, liquid helium) and of activated diffusion, and the biologist knows more about the physics of protoplasm, it is impossible to say how the diffusion of solutes is accelerated in the sieve-tube.

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pH DETERMINATION IN PLANT TISSUE

A. R. C. Haas

In a recent investigation[1] a procedure was found that permitted fairly accurate determinations of the pH of soils of low moisture percentages. One of the essentials of the method was the use of a horn spoon in spreading the moisture or soil films uniformly throughout the rather dry soil mass.

When plant tissue is finely cut in a grinder, relatively few of the cells are actually opened. The important fact is that the contents of the cut cells is smeared over the uncut groups of cells. On account of the somewhat analogous nature of the state of the soil and of the finely divided plant tissue, it appeared highly probable that the same methods that were applied in the determination of the pH of soils could be applied equally as well in the pH determination of plant tissue.

Materials and methods

The pulp of date fruits, the peel of lemon fruits, and the leaves of avocado seedlings furnished the material for the tests. A Beckman pH meter (model g) with a shielded glass electrode, and ten-foot extension cables attached to both electrodes, served as the means of measuring the pH.

The date fruits were wiped clean without being previously washed. The lemons and the avocado leaves were washed in running distilled water and then wiped dry. The date fruits were cut with a knife in order to discard the seed. The lemon fruits were peeled and the pulp was discarded, care being taken in the peeling process to avoid piercing the bounding membranes of the acid pulp. The samples were finely cut by means of a Universal food chopper, no. 3, making use of the finest of the four cutters. The chopper was used in place of the Wiley mill because of the relative ease in the washing and drying of the chopper, although most any cutting machine may be used. With very woody material the mill is most useful, although pencil sharpeners or whittling with a knife may be usefully employed.

The pH was determined immediately after each lot of material was cut in the chopper. The same sample of finely cut plant tissue that was used in the determination of pH was at once placed in a wide-mouthed glass jar that was tightly closed by means of a glass cover with suitable rubber gasket and metal clamp. As each sample was finished it was temporarily stored in a refrigerator until the group of samples was completed. Refrigeration was then obtained by storing the jars overnight in an underground freezing room of a cold storage company. The following day the jars were taken to the

[1] Haas, A. R. C. The pH of soils of low moisture content. Soil Science (In press).

laboratory where, after thawing the frozen plant tissue, the juices were expressed and the pH values determined in the extracted juices. Twenty thousand pounds pressure was used for the sap extraction from the date pulp and avocado leaves, while fifteen thousand pounds was used for the sap extraction from the lemon peel.

After the plant tissue was finely cut with the chopper it was thoroughly mixed in a shallow, wide glass dish. The juice from the cut cells was uniformly spread throughout the mass by means of a horn spoon which was used in the same manner as a spatula is used in mixing ointments. Heating of the material was overcome by working rapidly and by spreading out the material immediately after it emerged from the chopper. With some materials brief refrigeration may be of assistance in this regard. The cut tissue is placed in a beaker which is tapped on a folded towel on the table top. With the hand or any suitable tool, the tissue is well compacted within the beaker and the electrodes and thermometer are quickly inserted.

Both electrodes were held in an electrode holder provided with a spring clamp on a metal support. Upon squeezing the clamp the electrodes could be raised or lowered. The electrodes were pressed into the cut tissue and the surface tissue was firmly compacted about the electrodes in order to cover the unshielded portion of the glass electrode. A sufficient depth of well compacted tissue should occur between the bottom of the beaker and the sensitive part of the glass electrode. This material acts as a bumper or buffer in preventing the glass electrode from being broken against the bottom of the beaker. These electrodes are supposed to withstand pressures of thirty-five pounds and probably will withstand much higher pressures. In the inserting of the glass electrode into soils,[1] whenever care was taken to avoid contacting the bottom of the beaker, these electrodes withstood any slow steady pressures that could be applied with the hands.

After the temperature adjustment of the pH meter was made, the pH readings of the tissue were made. The electrodes then were more firmly pressed into the tissue and the surface tissue again was tightly compacted about the electrodes. Thus, pH readings were made a second time. This repetition or renewal of the electrode pressures and the surface compacting about the electrodes with the accompanying pH readings was continued until three successive repetitions of the process showed no appreciable change in the pH readings. This insures that the contact between the juice films on the tissue and the glass electrode is the most intimate that is obtainable.[1]

The sample of tissue was then removed as described and after being frozen, the sap was extracted under pressure. The pH of the juice was then determined and compared with that of the finely divided and freshly cut, but unfrozen, plant tissue.

Results

pH OF CUT FRESH FRUIT TISSUE AS COMPARED WITH THAT OF THE JUICE OF THE FROZEN TISSUE

A total of 100 unripe green dates were collected at Indio on July 24, 1939; the pulp weighed 158 grams and the seed 19 grams. The peel of seven ripe healthy lemons was used in the test with lemons. Table I shows the

TABLE I

pH OF FRESHLY CUT PLANT TISSUE AND OF THE SAP OBTAINED FROM THE FROZEN TISSUE

SAMPLE NO.	FINELY CUT FRESH PLANT TISSUE	JUICE EXTRACTED FROM FROZEN PLANT TISSUE
	DEGLET NOOR DATE PULP	
	pH	*pH*
1	5.55	5.45
2	5.65	5.47
3	5.55	5.41
4	5.60	5.50
5	5.62	5.50
Distilled water	5.27	
	PEEL OF RIPE HEALTHY LEMONS	
1	5.30	5.31

agreement between the pH values of finely cut fresh fruit tissue and those of the sap extracted from these tissues after being frozen. With date pulp which is very high in sugars there should be considerable opportunity for chemical changes to take place after the pulp is cut and yet the results were most promising, even in these preliminary tests.

pH OF FINELY CUT FRESH AVOCADO LEAVES AND OF THE JUICE EXTRACTED FROM THE TISSUE AFTER FREEZING

This experiment should not only serve to reveal whether the pH values of cut fresh tissue differ from those of the sap of the same sample of tissue after freezing, but should also indicate the effect, if any, of the pH of the soil upon that of the sap in plant leaves.

Hanford soil (pasture soil), obtained near the Citrus Experiment Station, was used in these cultures. An avocado seed was planted in each container of soil. The containers were six inches in diameter by seven inches high and contained 4000 grams of air-dry soil. Distilled water was used at all times. The cultures each received a total of 1.0947 grams of nitrogen applied in the form of calcium nitrate solution. This nutrient was divided into three equal applications to the soil during the growth of the cultures from May 1, 1939, to June 5, 1940. Various amounts of sulphur (table II) were applied to the surface of the soil after the tops were several inches high.

TABLE II

RESULT OF COMPARATIVE METHODS OF DETERMINING THE pH OF AVOCADO LEAF SAP OF PLANTS GROWN IN SOILS OF DIFFERENT pH

SULPHUR ADDED TO THE SURFACE OF SOIL CULTURES	SOIL SAMPLES AT END OF THE EXPERIMENT		MOISTURE PERCENTAGE OF THE SOIL AT END OF THE EXPERIMENT	FRESH WEIGHT OF LEAVES	FRESH WEIGHT OF TRUNK	FRESHLY CUT AVOCADO LEAVES	JUICE EXTRACTED FROM FROZEN AVOCADO LEAVES
	pH AT THE CULTURE SOIL MOISTURE PERCENTAGE	pH OF SUSPENSION OF OVEN-DRIED SOIL 1-5 SOIL-WATER RATIO					
gm.	*pH*	*pH*	%	*gm.*	*gm.*	*pH*	*pH*
0	6.42	6.86	10.1	55	64	5.45	5.47
0.05	6.53	6.72	10.3	85	101	5.47	5.50
0.10	6.49	6.84	11.8	69	90	5.41	5.44
0.50	5.90	6.37	11.0	86	132	5.40	5.40
1.00	5.26	5.95	8.2	88	106	5.43	5.42
1.50	4.82	5.42	6.9	98	104	5.55	5.57
2.00	4.72	5.04	9.1	91	104	5.40	5.37
2.50	4.39	4.83	6.8	89	99	5.63	5.70
3.00	4.21	4.55	6.6	98	102	5.80	5.98
3.50	4.31	4.46	6.8	65	77	5.63	5.61
4.00	4.02	4.24	10.6	16	36	5.65	5.76
4.50	3.80	4.10	10.6	Leaves dead	30		
5.00	3.75	4.11	11.7	Leaves dead	31		
	Soil samples divided into upper and lower halves						
0	6.10	6.70	8.0				
	7.27	7.38	8.1				
0.05	5.85	6.60	5.9				
	6.70	7.27	5.8				
0.10	6.05	6.66	7.8				
	6.82	7.24	8.3				
2.00	5.06	5.31	4.5				
	5.14	5.20	5.4				

Soil samples were obtained when the experiment was terminated. Six cores of soil, taken the full depth of the container, were used for the pH determination at the culture soil-moisture content. When oven dried, these soil samples were used to determine the culture soil-moisture percentage at the time of sampling and were also used for the pH determination of soil suspensions at the 1 to 5 soil-water ratio. A few days after the soil sampling, additional samples in a few containers were taken at the upper and the lower three-inch depths.

Table II shows the pH values of the soil when the experiment was concluded. In every case the pH values obtained in suspensions at the 1 to 5 soil-water ratio exceed those determined at the culture soil-moisture percent-

age occurring at the termination of the experiment. The pH at this latter moisture content more nearly represents the pH that affects the growth of these plants than does the pH at the 1 to 5 soil-water ratio.

In the soil samples (table II) taken from the upper portion of the soil mass, the pH at the culture soil-moisture percentage was less than that of the lower or deeper portion of the soil mass. Any acidity produced by oxidation of the sulphur or by the use of the distilled water (pH 5.30) brings acidity to the upper portion of soil before that to the lower or deeper portion.

Where no sulphur was applied the growth was poor. Even the smallest application of sulphur greatly improved the growth. With a sulphur application above 3 grams, growth was retarded, while at the 4.5- to 5-gram application, the leaves were dead and remained attached to the trunk which was alive.

Table II shows the range of soil acidity at the termination of the experiment. At or below pH 4.00 the growth was seriously retarded. The pH values for the leaves by the two methods of preparing the tissue agree very well. It is of interest that at the pH values of the soil (determined at the culture soil-moisture percentage) above pH 4.50, the pH values of the leaf tissue or juice are all approximately the same. Below this soil pH value, the pH readings for the tissue, or its juice, show a very slight tendency to be higher. Why the reaction of the tissues or their juices should tend to be less acid while the reaction of the soils are more acid, will require further study. At any rate, at the pH values which are comparable to those already found in orchard soils, the pH of the leaf tissue is notably constant.

In tables I and II it will be seen that the pH of the leaf or fruit tissue experimented with, was close to 5.5. Other pH determinations, for example those of the juice of the pulp of citrus fruits, are very much lower than this. With tissues other than these, as for example in the tissues of roots, very low pH values were also encountered. These pH determinations were made by whittling the root into many shavings which were treated in the manner described for cut fresh tissue. This technique should prove most useful for tissues from which it is difficult to obtain much juice, such as woody twigs, and for those tissues in which high air-suction or pressures are being avoided.

Summary

The method used for the pH determination in soils of low-moisture content was found to be equally applicable to plant tissue. The agreement between results obtained in this manner and those obtained with juice extracted from frozen tissue was most satisfactory. Over a wide range of pH in the soil of avocado cultures, no significant change in the pH of the leaf juice was noted.

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POTASSIUM DETERMINATION BY THE COBALTINITRITE METHOD AS AFFECTED BY TEMPERATURE AND pH

LELAND BURKHART

(WITH TWO FIGURES)

Rapid chemical analysis of plant and soil extracts for plant nutrients is being given widespread attention. Potassium is one of the most important and expensive plant nutrients and requires, therefore, special consideration of methods employed for determining its concentrations in soils and plant tissues.

The cobaltinitrite method for potassium determinations in extracts of soils and plants is becoming universal; many investigators, however, have not considered the importance of controlling temperature and pH during the precipitation reaction.

Early investigators noted the sparing solubility of the double salt prepared by precipitation of cobalt salts with strong solutions of potassium and sodium nitrite which led to the development of DE KONINCK'S reagent as a qualitative test for potassium. ADIE and WOOD (**1**) in gravimetric and volumetric determinations of potassium in fertilizers and soils by the cobaltinitrite method found it necessary to have relatively high concentrations of potassium (0.5–1 per cent.) in the precipitating medium in order to obtain reliable results. The precipitation of potassium salts in the sodium cobaltinitrite is appreciably more sensitive on addition of silver salts but this depends on the solution being halogen-free (**2**). Lithium, thallium, and ammonium salts must not be present as these also give crystalline precipitates with sodium cobaltinitrite. SCHUELER and THOMAS (**3**) report that it is necessary to keep the precipitating medium cooled to 5° to 8° C. for several hours in order to obtain satisfactory results.

In the course of developing a satisfactorily sensitive method for the rapid determination of potassium in extracts employing sodium cobaltinitrite, it was necessary to determine the effect of temperature over a wide range of potassium concentrations in order to establish a satisfactory set of conditions for the precipitation reaction. Potassium standards were prepared from potassium chloride. All solutions used were cooled in water baths to the respective temperatures indicated in figure 1 before adding the dry sodium cobaltinitrite. In preliminary trials it was found that sodium cobaltinitrite in the dry form is more sensitive than saturated solutions in equivalent amounts, even when the latter are accompanied by higher concentrations of sodium attained by addition of salts such as sodium nitrate and sodium chloride. Sodium cobaltinitrite in the dry form was used at a

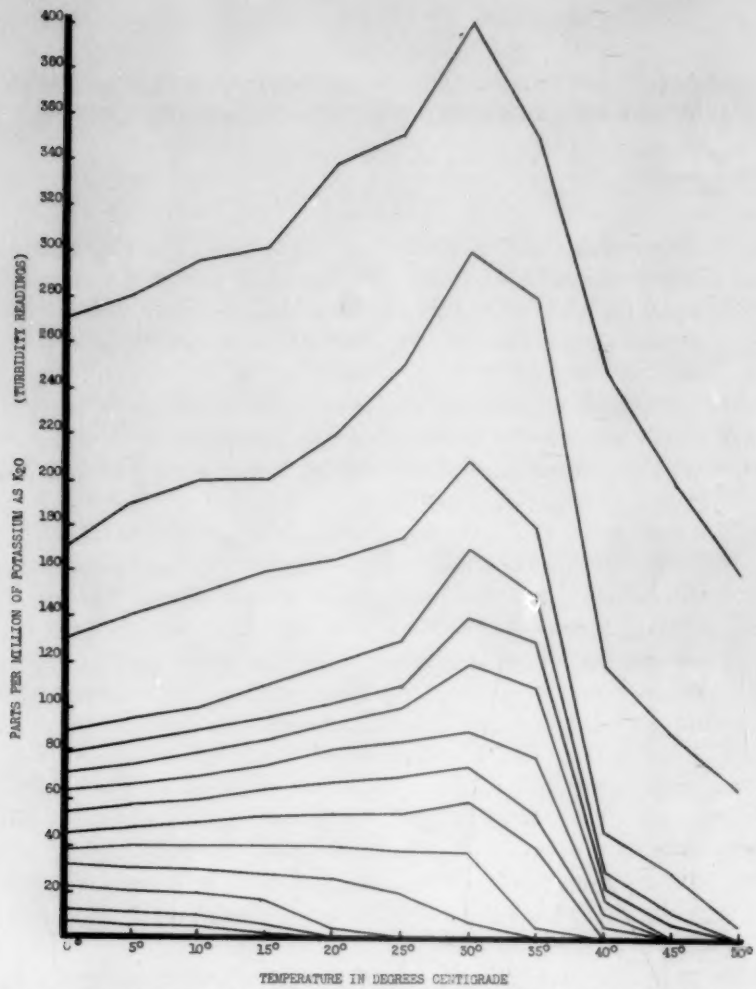

FIG. 1. Temperature of precipitating medium as affecting cobaltinitrite precipitation of potassium from prepared standards of potassium chloride. Turbidity readings at 10° C. used as standard of comparison.

rate to exceed 150 times the amount of potassium present in the precipitating medium.

To a pre-cooled 5-ml. portion of a potassium standard in a test tube there was added 60 mg. of sodium cobaltinitrite. Additional amounts of the reagent were used, however, at the rate of 60 mg. per 0.5 mg. of potassium when the concentration of potassium exceeded 100 p.p.m. After shaking the contents, 5 ml. of pre-cooled 95 per cent. ethyl alcohol were added and again shaken. One-half hour was allowed for the precipitation

and the turbidity compared with respective concentrations of potassium precipitated at 10° C. The turbidity developed by precipitation of various concentrations of potassium at 10° C. was used as a standard of comparison.

The results of the precipitations of the various concentrations of potassium at the respective temperatures are given in graphic form in figure 1. Of special significance is the fact that at low concentrations of potassium (5 to 20 p.p.m.) no precipitate is formed at or above temperatures of 20° C. Many of the extracts of productive peanut soils in North Carolina have concentrations of potassium which are less than 20 p.p.m. and it is, therefore, imperative that a low precipitating temperature be maintained when estimating low concentrations of potassium. At concentrations higher than 40 p.p.m. more precipitation occurs at 25° to 30° than at 10° C. Temperatures above 30° C. result in marked decreases in precipitation at all concentrations of potassium. It was found that the precipitation at 10° C. results in a much wider readable range of potassium concentrations. It is evident

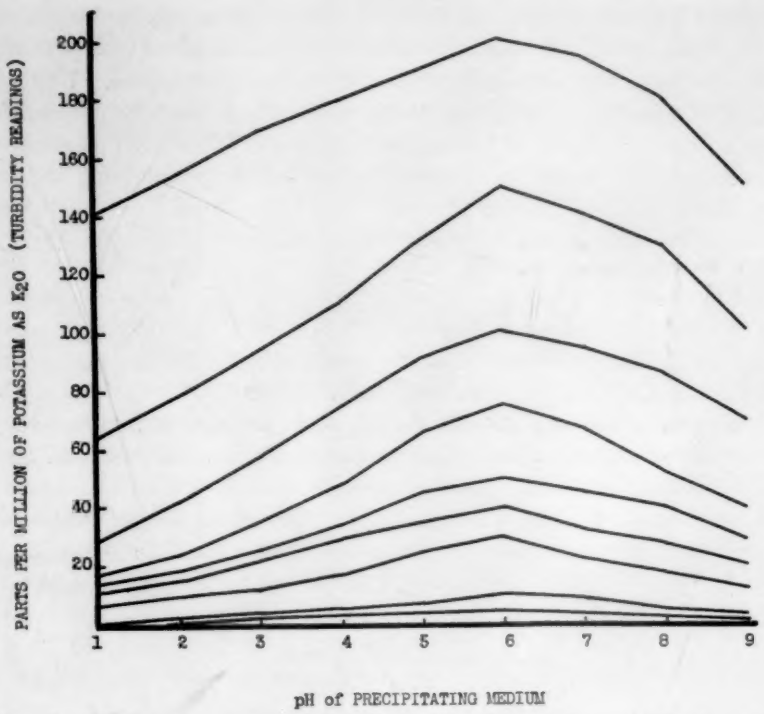

FIG. 2. Hydrogen ion concentration of precipitating medium as affecting cobaltinitrite precipitation of potassium at 10° C. from prepared standards of potassium chloride made up in respective buffer systems. Turbidity readings at pH 6 used as standard of comparison.

that the rapid determination of potassium by turbidity readings of cobaltinitrite necessitates controlling the temperature of the precipitating medium.

The influence of the hydrogen-ion concentration of the precipitating medium is shown graphically in figure 2. The respective pH buffer systems employed were as follows: pH 1 and 2, NaCl + HCl; pH 3 to 6, AcOH + NaOH; pH 7, combination of pH 6 and 8; pH 8 to 12, H_3BO_3 + NaCl + NaOH. These systems were made up from normal solutions to the desired pH by the electrometric titration procedure employing the glass electrode and a potentiometric pH meter. The phosphate buffer system could not be used because of interference of high concentrations of phosphate with the cobaltinitrite precipitation. As shown in figure 2 a buffer system at pH 6 is the most sensitive, therefore desirable as a precipitating medium for the estimation of all readable concentrations of potassium. Under alkaline conditions above pH 9 decomposition of the cobaltinitrite makes it impossible to determine potassium by this method.

From these results it appears that the precipitating medium should be maintained at 10° C. and pH 6 for the rapid determination of potassium by the cobaltinitrite method. Potassium standards should be prepared in buffer systems at the same pH as the extracts being examined. Under these controlled conditions of temperature and pH, a readable concentration range of 2 to 200 p.p.m. of potassium is attained. Turbidity measurements are satisfactorily made by the photoelectric method when using the proper color filter.

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CHANGES IN CHEMICAL COMPOSITION OF TWIGS AND BUDS OF YELLOW POPLAR DURING THE DORMANT PERIOD

J. Joseph McDermott

(with two figures)

Introduction

In order to study the chemical changes that accompany the breaking of dormancy in yellow poplar, *Liriodendron tulipifera* L., upon treatment with ethylene chlorohydrin vapor, it was necessary to determine what changes occur under natural conditions. It is with these latter changes that this paper is concerned.

Methods

During the dormant period a series of five samples of the buds and of the preceding year's growth was taken from a number of dominant and codominant yellow poplar trees. The samples were dried in an oven at 65° C., and were then ground in a ball mill until the material would pass through a 100-mesh sieve. Duplicate samples of from 25 to 50 grams were taken from this large sample, covered with 80 per cent. (by volume) aldehyde-free ethyl alcohol, and allowed to stand overnight. The liquid was decanted off and the last portion filtered through a hardened filter paper. The residue was dried at 105° C., coarsely pulverized, and extracted in a Soxhlet extractor until the percolate was colorless. The two extracts were united and made up to 1 liter with 80 per cent. alcohol. The residue from the last extraction was dried, coarsely ground, and preserved for analysis.

Reducing sugars were determined by the Shaffer-Hartman method in a 200-ml. aliquot of the extract. Total sugars were determined in the reducing sugar aliquot after hydrolysis with HCl at 73° C. for ten minutes.

Total soluble nitrogen was determined in 50-ml. aliquots of the extract, and total insoluble nitrogen on 1.0-gram samples of the residue by a modification of the Kjeldahl method.

A 1.0-gram sample of the residue was refluxed with 5 per cent. HCl for four hours, and the reducing value of the solution (as glucose) was reported as total reserve polysaccharides. Starch was determined by incubation at 38° C. of 2.0-gram samples of the residue suspended in 200 ml. of water with 0.1 gram of taka-diastase. Reducing sugars were determined on the solution.

Results

The analytical data obtained are assembled in table I and are graphically shown in figures 1 and 2. In explanation it should be stated that when

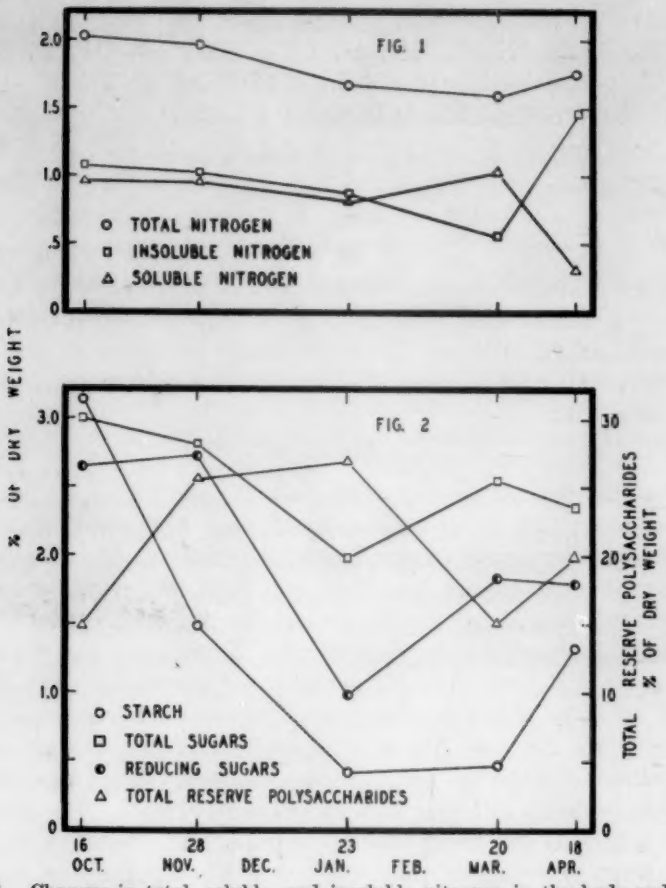

FIG. 1. Changes in total, soluble, and insoluble nitrogen in the buds and one-year-old twigs of yellow poplar during the fall and winter of 1936–37.

FIG. 2. Changes in reducing sugars, total sugars, starch, and total reserve polysaccharides in the buds and one-year-old twigs of yellow poplar during the fall and winter of 1936–37.

sample 5 (April 18) was taken, the buds were just breaking open and some stem elongation had occurred. Some leaves had appeared, and they were about a centimeter in breadth.

During the period from October 16 to January 23 there was a gradual loss in organic nitrogen. This may be a real loss of nitrogen from the twigs and buds, or, more plausibly, merely the dilution of the organic nitrogen present by the accumulation of increasing amounts of reserve polysaccharides. The small gain in the total nitrogen shown for the period from March 20 to April 18 might be attributed to the movement of soluble nitrogenous compounds from the older twigs and branches into the expanding

TABLE I

PERCENTAGE COMPOSITION OF BUDS AND ONE YEAR OLD TWIGS OF YELLOW POPLAR ON A DRY WEIGHT BASIS

FRACTION	DATE				
	OCT. 16	NOV. 28	JAN. 23	MARCH 20	APRIL 18
	%	%	%	%	%
Soluble nitrogen	0.96	0.95	0.81	1.03	0.31
Insoluble nitrogen	1.07	1.02	0.87	0.56	1.46
Reducing sugar	2.65	2.73	0.98	1.84	1.80
Total sugars	2.99	2.81	1.98	2.55	2.36
Starch	3.14	1.49	0.41	0.46	1.34
Total reserve polysaccharides	14.85	25.61	26.89	15.11	19.91

buds (3). During the period from January 23 to March 20 there was a conversion of much of the insoluble nitrogen into more soluble forms. In the period from March 20 to April 18 these soluble compounds were converted into insoluble compounds, probably proteins for the production of new protoplasm in the cells of the rapidly expanding buds.

All of the carbohydrate fractions with the exception of the total reserve polysaccharides seem to be used in respiration. When the sugars and starch were reduced materially in amount, there was a decrease in the amount of total reserve polysaccharides of about 10 grams *per centum*, and at the same time there is an increase in the amount of total sugars of only 0.5 grams *per centum*. Since the trees were dormant at this time, any photosynthetic activity by the chlorophyllous tissue of the buds would not be sufficient to account for any increase in dry weight which might cause such an apparent decrease in the total reserve polysaccharide content. The only tenable hypotheses are that some of these complex carbohydrates or derivatives of them were utilized in the respiratory processes of the twigs and buds, or that they were converted into soluble compounds which were then transported to other portions of the plant. It is also possible, as suggested by O'DWYER (4) that the hemicelluloses are intermediate compounds in the synthesis of lignin from the pectic compounds, and this loss of hemicelluloses might then be attributable to a continuing lignification of the woody tissues. The utilization of the hemicelluloses as a reserve carbohydrate has previously been reported by JONES and BRADLEE (1) in sugar maple, and by MURNEEK (2) in apple spurs. WINKLER and WILLIAMS (5), however, were not able to demonstrate the metabolic utilization of hemicelluloses in shoots of grape vines dying because of repeated defoliation. The latter investigators concluded that hemicellulose probably functions as a structural material and not as a reserve food material in grape shoots. In the period between March 20 and April 18 the small leaves extending beyond the bud

scales were probably active photosynthetically, as is evidenced by the formation of starch and by increase in the amount of hemicelluloses during the period.

Summary

1. Some of the chemical changes occurring in the buds and one-year-old twigs of yellow poplar during the winter, and on breaking of dormancy are reported.

2. There was an apparent decrease in total nitrogen. During February and early March much of the protein was converted into more soluble compounds which were resynthesized into insoluble forms, probably proteins for the production of new protoplasm in the expanding buds.

3. All of the carbohydrates appear to be used in respiration, starch and the sugars most readily, and the hemicelluloses least readily, if at all. With the resumption of photosynthetic activity starch appears, and the hemicelluloses are synthesized again.

The writer is indebted to Dr. PAUL J. KRAMER for many helpful suggestions during the course of this study.

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BRIEF PAPERS

A PRESS FOR RECOVERY OF FLUIDS FROM PLANT TISSUES[1]

T. C. Broyer and A. H. Furnstal

In the course of research upon expressed plant tissue fluids a press was constructed incorporating features of earlier models used in our laboratory. Since this apparatus may be of interest to other investigators, we present a detailed reproduction of the design (fig. 1).

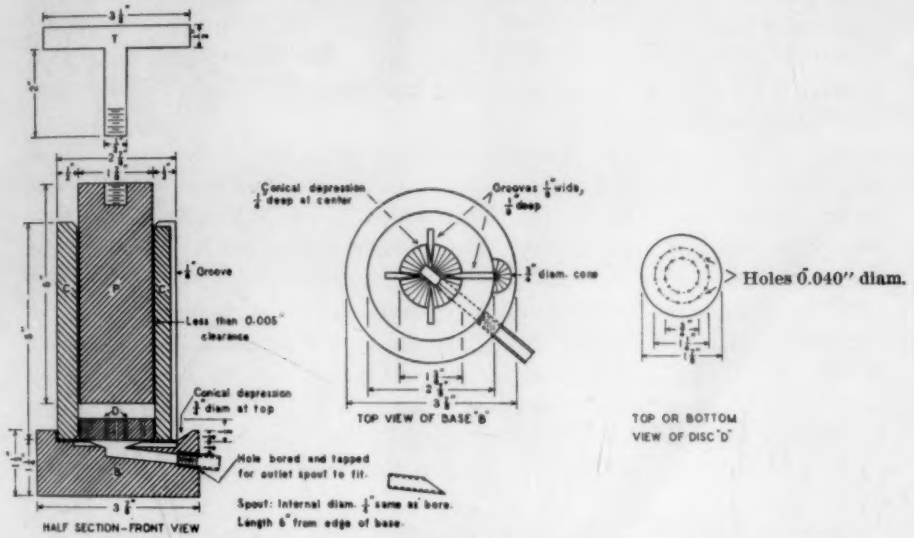

FIG. 1. Details of press for recovery of fluids from plant tissues. B, base; C, cylinder; D, disc sieve plate; P, piston; S, delivery spout; T, T-rod for piston withdrawal.

The dimensions given are suitable for a press to contain about 25 grams of tissue. For larger samples, the diameters of the piston, cylinder, disc sieve plate and base, and thickness of cylinder wall may be increased proportionately. The height of the press should be limited in order to minimize possible internal resistances to compression of the tissues themselves (1). Increase in height may also be limited by the stroke length of the manual screw or working stroke of the hydraulic jack employed for applying pressure.

It is recommended that stainless steel block and tubing be used for all

[1] Clerical assistance in the preparation of this publication was furnished by the personnel of Work Projects Administration Official Project No. 65-1-08-91-B-10.

parts. Materials with chemically resistant characteristics minimize the solution of metals by the expressed fluids. The metals might prove deleterious in subsequent use of these fluids. To avoid electrolytic action between the component parts of the system, dissimilar metals (as steel and brass) should not be used.

The clearance between the plunger and cylinder is of necessity small. The piston may increase in diameter under compression unless made from very hard materials. If the resistance of the steel to penetration is not sufficiently great and if the elastic limit is exceeded certain component parts of the press may become "frozen" to one another. It is essential, therefore, that those parts which are subject to pressure or motion, or those adjacent to moving parts, be fabricated from metals of likewise favorable physical characteristics.[2] In order to reduce the hazard of the plunger becoming frozen to the cylinder, it is obviously necessary that the pressure be applied directly perpendicular to the piston and that the tissue sample be uniformly distributed within the press.

The delivery spout is removable from the screw tap in the base. When fully inserted it should fit flush with the drilling of the base, and the end taper should then be in the upright position. The thickness of the base should be such that the delivery spout clears any object upon which the base is placed.

If the piston can not be manually removed from the assembly it may be withdrawn with the aid of a T-rod inserted in the threaded tap in the top of the piston (**2**). If, however, the pressure is applied from above by means of a screw without an attached platen base, the threaded tap in the piston may be omitted in the construction of the plunger and a hard-metal cupped bearing may be inserted between the screw and the piston during operation.

As shown earlier (**1, 2**), filters of cheese cloth or muslin enclosing the tissue or inserted as a disc between the sample and the sieve plate aid in the expression of a clear relatively non-colloidal fluid.

In order to recover all of the available liquid expressed, we have avoided the insertion of a sap groove in the cylinder wall and tap hole in the sieve plate. The press assembly is so arranged that it may be conveniently and safely tipped, at the conclusion of pressing, to allow complete drainage of sap.

A heavy wire ram-rod covered with cheese cloth may be employed to dry the delivery spout between samples. The channels in the sieve plate are easily cleaned of compacted tissue residues by reaming out with an ordinary paper clip or needle. All parts are dried with moderate heat

[2] "U. S. S. chromium-nickel alloy steel 18–8" has been suggested as possessing favorable chemical and physical characteristics.

and stored in a closed chamber to prevent corrosion by fumes from the laboratory.

The authors are indebted to several members of the Division of Plant Nutrition for suggestions relative to the construction of this instrument.

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SEED IMPRESSIONS ON PLASTIC FILMS

THORA M. PLITT

(WITH ONE PLATE)

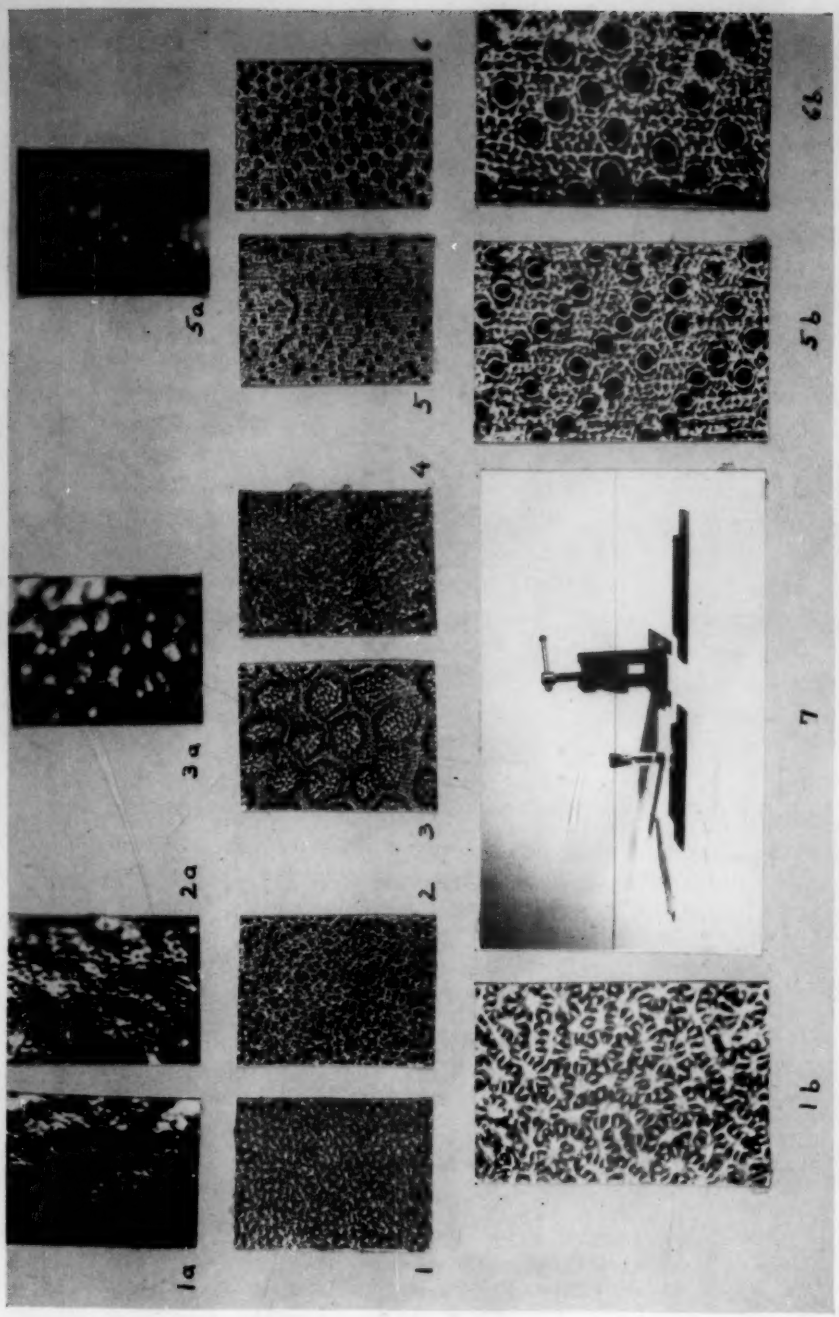
Microscopic details on the hard surfaces of seeds or other parts of fruits are frequently sufficiently characteristic to permit the accurate identification of related species. These taxonomic features have hitherto been largely neglected, however, because of the difficulty of seeing them directly under the microscope. The thickness of the fruit structures, the presence of pigmentation, and the reflection of light from smooth surfaces are difficulties that may be overcome by making impressions of the structures on plastic films. This new method is an outgrowth and modification of a method of obtaining impressions of the surface structures of fur fibers recently developed by HARDY and PLITT (1).

Seeds were placed in carbitol acetate[1] in a small dish for a minute or two. They were then removed by means of forceps and placed between two pieces of transparent cellulose acetate film, 0.005 inch thick and 1 inch square. Adequate padding to obtain clear impressions without crushing the seeds was built up by placing on each side of this double stratum the following: 4 additional strips of plastic film, one strip of sponge rubber (insulation stripping) approximately 0.25 inch thick, and a smooth steel plate. The whole stack was held together by means of a wide clamp which was screwed down until the rubber was compressed to $\frac{2}{3}$ or $\frac{1}{2}$ of its original thickness. This unit was placed for 5 minutes in an electric oven maintained at approximately 90° C. Upon removal from the oven the central double stratum containing the seeds was taken out of the unit and allowed to cool and harden before removing the seeds with a brush or needle. The two plastic films bearing the impressions were mounted face down on glass slides with very narrow strips of transparent adhesive film around the edges. Thus the impressions are positive images of the surfaces. The impressions are best viewed through the microscope using oblique transmitted

[1] Carbitol acetate may be obtained from the Carbide and Carbon Chemical Corporation, 30 E. 42d Street, New York, N. Y.

PLATE V

Photomicrographs of impressions (100×) of the achenes of (1) *Polygonum punctatum*, and (2) *P. persicaria;* of the seeds of (3) *Brassica juncea*, and (4) *B. napus;* and of the lemmas of (5) *Poa compressa*, and (6) *P. pratensis*. Photomicrographs of the corresponding parts of the fruits taken directly (100×): (1a) *Polygonum punctatum*, (2a) *P. persicaria*, (3a) *Brassica juncea*, (5a) *Poa compressa*. Enlargements of the above impressions (300×): (1b) *Polygonum punctatum*, (5b) *Poa compressa*, and (6b) *P. pratensis*. (7) Photograph of equipment used in making impressions.


1a
1
2a
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light, with an amber filter. The 10× objective has considerable depth of focus; magnifications of 100 to 300 diameters are obtained by interchanging suitable oculars.

In this procedure the purpose of placing the seeds in carbitol acetate is merely to use a minimum quantity of the liquid to soften the film. Variations in the procedure for convenience may easily be introduced to suit the material or the equipment at hand, such as the thickness of film, thickness of padding, time, temperature, and manner of controlling the temperature.

The accompanying illustrations show the microscopic details of some seeds or other fruit structures. These materials were furnished by the Section of Food Habits of the Fish and Wildlife Service. The three sets of related species were selected because of their similarities and attendant difficulties of identification, because of their importance in food-habit studies, and in agricultural problems. It may be noted that unsuspected details are revealed by the impressions, and furthermore that the arrangement of characteristic features is visible over a greater area than by direct inspection of the seeds. Clear definition of fine details at higher magnifications may be obtained by use of the impression method. Patterns once established at high magnifications are subsequently more readily recognized at lower magnifications.

This method is applicable in taxonomic studies, in the determination of certain foods and drugs, and in the identification of difficult seeds and fruits in food-habit studies. Impressions may be obtained in a few minutes; furthermore many impressions may be made simultaneously on one set of films.

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COMPARATIVE TRANSMISSION SPECTROGRAMS OF DIFFERENT CONCENTRATIONS OF LEAF EXTRACT

LEWIS W. WEBB, JR. AND FREDERICK F. FERGUSON

(WITH THREE FIGURES)

This study continues the work upon the physical properties of leaf extracts and is a preliminary effort to show the effects of increased dilution of an alcoholic solution of leaf green. FERGUSON, DE LOACH, and WEBB (*loc. cit.*, p. 560, fig. 2) have indicated that dilution of the ethyl alcohol stock solution produces changes in the transmission spectrogram giving an increase in both transmission and absorption regions.

The stock solution used in this study was prepared in the following manner: methyl alcohol (100 ml.) was added to selected fresh green leaves of *Poa pratensis* (3 gm.) and the resulting mixture was heated for 20 min. on a water bath under a reflux condenser. The rather dark green alcohol solution was then decanted. This stock solution considered as 100 per cent. (curve no. 1, fig. 1) was then diluted with methyl alcohol giving these percentages: 50 per cent. (curve no. 2), 25 per cent. (curve no. 3), 10 per cent. (curve no. 4), and 1 per cent. (curve no. 5). The Coleman Regional Spectrophotometer was used to obtain all graphs in the study. The points recorded in each curve represent the average transmission of bands 30 mμ in width.

It may be noted that the form of the curve of transmission of an ethyl alcohol solution of leaf extract is essentially the same as that given by the stock solution (curve no. 1) of this study. There is a tendency to adhere to this characteristic form as shown by the five curves of figure 1. There is relatively little transmission in the region of 350 mμ to 450 mμ in solutions of 100 per cent., 50 per cent., and 25 per cent. Transmission in this area increases sharply in the 10 per cent. solution which displays a marked inclination at *ca.* 430 mμ. Curve no. 5 shows a relatively higher percentage of transmission for the region of *ca.* 350 mμ to 450 mμ than do the other curves. As is expected, the transmission peak at *ca.* 520 mμ increases as the solution is diluted. Curve no. 5 yielded by the 1 per cent. dilution, which was so weak as to be visually indistinguishable from methyl alcohol, still maintains some semblance of this peak. As dilution increases the characteristic "absorption band" as shown by the sharp inclination at *ca.* 660 mμ becomes less accentuated. Curve no. 5 retains but small evidence of this major feature. All curves agree in their sharp inclination at *ca.* 660 mμ and in the leveling off in the infra-red.

The minimum transmission of 0.4 per cent. appears at *ca.* 360 mμ (curve no. 1) while the maximum transmission of 99.6 per cent. appears at *ca.* 740 mμ (curve no. 4). The spectral range extends from 360 mμ to 950 mμ.

In analyzing the comparative graphs shown in figure 1, the peak, at *ca.* 530 mμ, was explained. It was found that the rate at which the transmis-

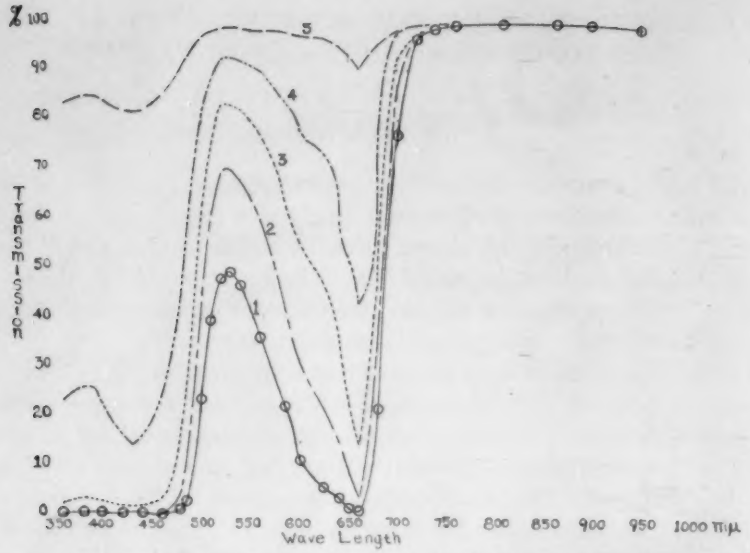

FIG. 1. Comparative graphs showing effects of dilution upon the transmission spectrograms of alcoholic leaf extracts.

sion increases in respect to the decrease in concentration of the solution follows a well defined exponential equation.

Equation no. 1

where: T = percentage of transmission
D = percentage of concentration
e = Naperion base

$$T = 99.65\ e^{-.007\ D}$$

The following figure represents this graphically:

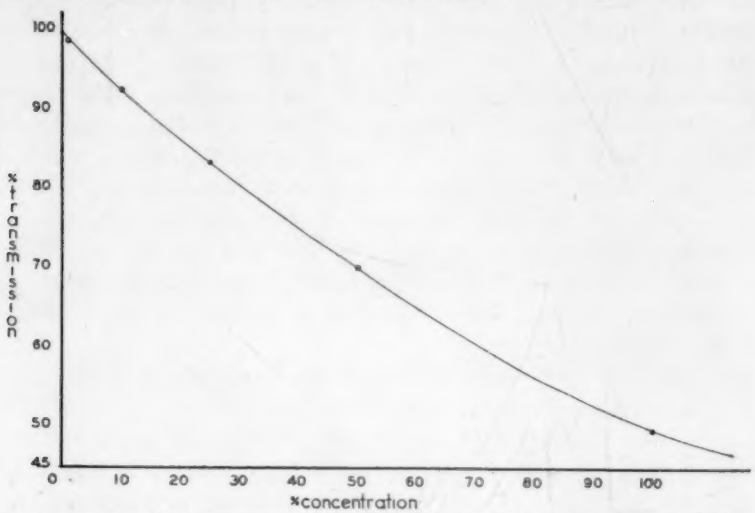

FIG. 2. Graph showing increase in transmission with decrease in concentration of a solution of alcoholic leaf extract for *ca.* 530-mμ wave band.

The trough of the graphs of figure 1, at *ca.* 660 mμ, was also studied. It was ascertained that the rate at which transmission increases in respect to the decrease in concentration follows a hyperbolic equation.

Equation no. 2

$$(d + 8.6)(T + 7.3) = C = 926$$

where: T = percentage of transmission
D = percentage of concentration
C = constant

The following figure shows this relation graphically:

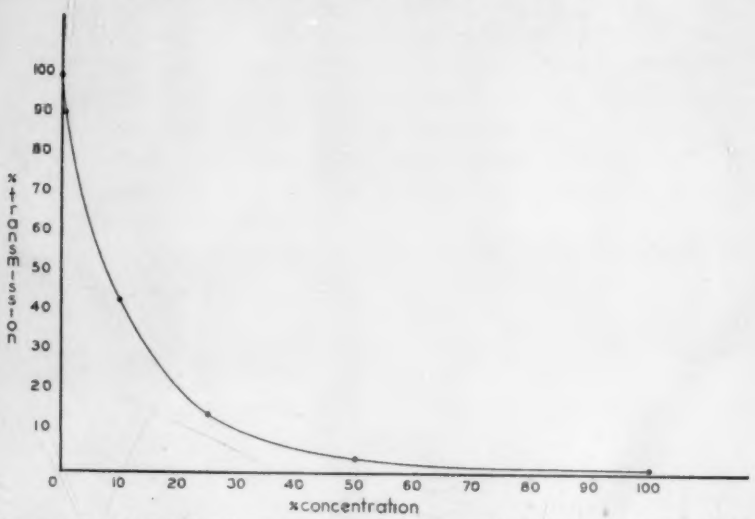

FIG. 3. Graph showing increase in transmission with decrease in concentration of a solution of alcoholic leaf extract for *ca.* 660-mμ wave band.

Further comparative studies on the effect of dilution upon leaf extracts are considered by the authors.

COLLEGE OF WILLIAM AND MARY—VIRGINIA POLYTECHNIC INSTITUTE
NORFOLK DIVISION AND
U.S.P.H.S. CRANEY ISLAND LABORATORY

LITERATURE CITED

1. FERGUSON, F. F., DELOACH, W. S., and WEBB, L. W., JR. Transmission spectrogram of leaf extracts. Plant Physiol. 15: 559–560. 1940.

NOTES

Southern Section.—The second annual meeting of the Southern Section of the American Society of Plant Physiologists was held at Atlanta, Georgia, on February 5 and 6, 1941, with the Association of Southern Agricultural Workers. The 1942 meeting will be held in Memphis, Tennessee. The attendance was very gratifying, for the increase over the first meeting in 1940 was over 300 per cent. Forty-five were present, there were three sessions instead of one, and 23 papers were read. Membership has more than sextupled during the year, as there are now about 75 members.

These signs of vigor and responsiveness of the southern scientific men to their opportunities are encouraging to the officers who have been responsible for the early steps of this growing organization. The prospects are that the section will grow and aid in a most energetic expansion of research in the South, which is now ready to move forward as the most rapidly developing region in the United States.

A high spot in the program at Atlanta was a round table discussion arranged by Dr. I. E. MILES, of Raleigh, North Carolina, on "The General Aspects of Plant Nutrition." The theoretical aspects were discussed by Dr. J. R. JACKSON, Auburn, Alabama; the general application of these problems in agriculture by Dr. L. D. BAVER, Raleigh, N. C.; the application to horticulture, by Dr. E. M. EMMERT, Lexington, Kentucky; and the commercial aspects, by Mr. SAM F. THORNTON, Norfolk, Virginia.

Officers for 1941–1942 were elected as follows: Dr. L. H. FLINT, Baton Rouge, Louisiana, Chairman; Mr. SAM F. THORNTON, Norfolk, Virginia, Vice-Chairman; Dr. C. F. MORELAND, Baton Rouge, Louisiana, Secretary-Treasurer.

The section can now look forward to ever-increasing influence in the development of research in a potentially rich agricultural region.

New England Section.—The New England Section has deferred its customary May meeting in order to join with the parent society which holds its summer meeting this year at the University of New Hampshire, Durham, N. H., June 25–26, 1941. This institution is celebrating the seventy-fifth anniversary of its founding, and is anxious to extend its hospitality to plant scientists. June is a pleasant month in New England; college classes will be over; plan to meet your friends in Durham, and relax in the Granite State. Information about the meetings, and accommodations for visitors may be obtained from Dr. T. G. PHILLIPS, the University of New Hampshire, Durham, New Hampshire. It is hoped that there may be a large group of visitors from outside regions who desire to know their New England colleagues better, and to see their fine facilities for research.

Western Section.—The Western Section will hold its annual meeting for 1941 with the Pacific Division of the A. A. A. S. at the California Institute of Technology, Pasadena, California, from June 16 to June 21. Scheduled are symposia on Nutrition (chairman Dr. D. R. HOAGLAND); Plant Hormones (chairman Dr. F. W. WENT); Protoplasm (chairman Dr. O. L. SPONSLER). These symposia are invitational programs, and will be held jointly with other societies. An excursion to the Rancho Santa Ana Botanic Garden, and another to the U. S. Regional Salinity Laboratory at Riverside, California, have been planned. Approximately three and one-half days have been set aside for submitted papers. Titles should reach Dr. J. VAN OVERBEEK, secretary of the Western Section (California Institute of Technology, Pasadena), not later than the end of April.

Jethro Tull Portrait.—It is a great pleasure to present our readers with the fine portrait of JETHRO TULL in this number of PLANT PHYSIOLOGY. It was printed fifty years ago by the Royal Agricultural Society of England. It was made from a contemporary painting which at that time, 1891, was in the possession of Mr. MARTIN J. SUTTON. The painting has had an interesting history. It was possibly painted by ALLAN RAMSAY, a contemporary painter who knew of TULL and his work. It was owned a century ago by Mr. JOHN RICHARDS, of Camden House, Reading. In 1840, at the time Mr. RICHARDS died, the portrait was purchased by Mr. JOHN SNARE, of Reading; in turn his widow sold the portrait to Mr. SUTTON, who has preserved it in the public interest. Speaking of this portrait, EARL CATHCART describes it in the following words: "Fancy him at the age of fifty-six . . . arrayed in his best ruffled velvet coat and full-bottomed wig; and having dined well, he is depicted as demonstrating, with index finger extended and self-satisfied expression, the excellences of some excellent part of his agricultural system —a charming discourse, spiced no doubt with humor, and delivered with the ease of a scholarly gentleman, together with the dignity of an intuitive philosopher." This seems to be a very good interpretation of the portrait, and one can almost hear TULL discussing his hoeing husbandry, with persuasive and authoritative emphasis.

Errata.—Attention has been called to some errors in the issues of 1940, and we are glad to report these to our members, with thanks to those who discovered and reported them. Please note the following:

P. 468, line 2, for "*Elyonurus,*" read *Elymus.*
P. 484, citation 9, for "*Elyonurus,*" read *Elymus.*
P. 545, citation 3, for 93:, read 63:.
P. 719, line 9 from bottom, for "(8)," read (7).
P. 719, line 2 from bottom, for "(7)", read (6).
P. 720, first paragraph on preparation of reagents, read as follows: Fehling's solu-

tion A was prepared according to MUNSON and WALKER (3); solution B was prepared according to the modification proposed by QUISUMBING and THOMAS (5) as follows:

P. 124, line 6, for "precipitate with," read precipitate. With

P. 724, line 7, for "bottle add," read bottle, break up the precipitate, centrifuge, and decant excess wash water. Add

P. 725, citation 3, for "THOMAS," read WALKER.

P. 771, line 3 from bottom, for "English," read French.

Fellowship for Women.—The announcement of a new fellowship for women, offered by Sigma Delta Epsilon, Graduate Women's Scientific Fraternity, reached us too late to be announced in the January number of PLANT PHYSIOLOGY. As the award would be made before this announcement could reach our members, it is mentioned now mainly to draw the attention of our members to this enlightened and courageous undertaking by Sigma Delta Epsilon. This splendid organization is celebrating its 20th anniversary, and is saying it by thinking of others, perhaps in memory of their own struggles to obtain adequate opportunities for research after the Ph.D. degree had been obtained.

The completion of this project under Dr. LOIS LAMPE, of Ohio State University, National President in 1940, is followed by consummation under the presidency of Dr. DOROTHY DAY, of Smith College, National President in 1941. The stipend mentioned in the announcement is $1000 to $1500. The holder must have the equivalent of the Ph.D. degree, and pursue research either in mathematical, physical, or biological science. She must devote her entire time to the approved research project, or obtain written approval of the Board for any deviation from this obligation.

Sigma Delta Epsilon is the only women's organization affiliated with the A. A. A. S., and is represented by 14 chapters, with about 2500 members. The organization has a right to be proud of its accomplishments, and is setting a challenging example of unselfish devotion to science, and to the welfare of women in research. They deserve hearty congratulations and commendation for their efforts.

Photosynthesis.—It is 15 years since a monograph on photosynthesis has appeared. During this long period much progress has been made, especially in the chemical study of chlorophyll, methods of analysis, and physical-chemical approaches to the problems of carbohydrate synthesis by plants. We are now presented with a monograph by E. C. C. BALY, Emeritus Professor of Chemistry in the University of Liverpool. It bears the simple title *Photosynthesis*, and is published by D. Van Nostrand Co., 250 Fourth Ave., New York. The work records Professor BALY'S experiences and deductions from a long series of studies involving concepts of physical chemistry. It deserves, and will no doubt receive, critical examination by

all workers engaged in this field. BALY claims to have brought about a genuine photosynthesis *in vitro*, and there has been some criticism of his methods and deductions while his papers were appearing. In this welcome volume we now have a full and mature statement of his views, based upon his researches over a long period of years. Naturally it raises the whole question of whether one could assume, even if *in vitro* synthesis were accomplished, that it shed any light upon the naturally occurring processes in leaves.

There are eight chapters, with titles as follows: Photosynthesis and the difficulties of its interpretation; the action of ultra-violet light on aqueous solutions of carbon dioxide; the polymerization of formaldehyde into glucose; the photosynthesis of carbohydrates by the action of light on hydrated carbon dioxide adsorbed on a surface; the final achievement of photosynthesis of carbohydrates; the assimilation of nitrogen by the living plant; the mechanism of photosynthesis; and the kinetics of photosynthesis. There are brief author and subject indices.

It is written in very readable style, and should stimulate others to work on these phases of the synthetic processes. It is gratifying to have this summation of Professor BALY'S life work, all brought together in a compact statement, where it can be viewed as a whole. Plant physiologists will want to read it carefully and thoughtfully. It is a work of 248 pages, with 24 illustrations, and may be obtained from the publishers at $4.25 per copy.

Enzyme Research.—Lieferung 5 of this monumental work, *Die Methoden der Fermentforschung*, has been received from the press of Georg Thieme, Leipzig. It contains 560 pages, and the "Auslands" price for this number is R.M. 42. Its contents may be briefly summarized, as follows: Active enzyme preparations from algae, yeasts, and molds; from lower animals, particularly the one-celled animals, and from both animal and plant tissues.

The monograph then takes up the isolation and characterization of lyo- and desmo-enzymes; general procedures for concentrating enzymes and for their separation from one another; and the determination of certain general properties of enzymes. These include the splitting of enzymes into their components; direct and indirect proof of the presence of certain atomic groups in enzymes; and the affinities of enzymes for their substrates, reaction products, and other substances.

An appendix to part I, which is concluded here, deals with the effects of radiations on enzymes, and enzyme reactions in heavy water.

The second major division of the work (Zweiter Hauptteil: Spezieller Teil) begins on page 1547. It takes up first the hydrolases, which include the following: Lipases, cholinesterase, tannase, chlorophyllase, phosphatases

(both plant and animal), phytase, lecithase, and sulphatases. The stereochemical specificity of ester-splitting enzymes, and the synthesizing effects of ester-splitting enzymes come in for treatment at the close of the hydrolases of the esterase type.

The last portion of Lieferung 5 takes up the carbohydrases. There is a general treatment of these, then saccharase and invertin, maltase, b-glucosidase, galactosidases, trehalase, digilanidase and other specific glucosidases, heteroglucosidases, glucuronidase, and thioglucosidases (myrosin group) complete the 5th Lieferung.

This brings the total work to page 1836, about half of it, or possibly more than half. BAMANN and MYRBÄCK are making a great contribution to science in providing such a source book of information. Enzyme workers of the world will owe them a debt of gratitude for bringing out this excellent compendium on enzyme research.

Mineral Deficiencies.—Plants are now known to exhibit symptoms of mineral deficiencies in widely scattered areas throughout the United States. While the symptoms are more frequently observed in regions of loose soils and heavy rainfall such as the coastal plains, almost any region may show deficiencies of elements needed in mere traces. It has become necessary for the physiologist to recognize these symptoms at sight, and to be on the lookout for them everywhere. The prompt recognition of specific responses to specific deficiencies is important, and any helps toward this end are important. Under the title: *If They Could Speak*, the Chilean Nitrate Educational Bureau, Mr. HERBERT C. BREWER, Director, 120 Broadway, New York, has issued a booklet with many color reproductions of plant mineral deficiency symptoms. They were made from Kodachrome transparencies, and truly, the plants speak for themselves. There are 95 reproductions, two per page with one on the front cover, and they carry brief explanatory legends. The deficiencies illustrated concern magnesium, boron, manganese, copper, zinc, iron, potash, calcium, and nitrogen. About 35 crops are included in the survey, each showing one or more deficiency diseases. Some of the more important crops are cotton, corn, oats, tobacco, apples, citrus fruits, soy beans, tomatoes, cabbage, cauliflower, celery, etc.

This excellent educational booklet, 54 pages, can be obtained free on request from the director of the Bureau. It should be in the hands of every plant physiologist. Mr. BREWER would also like to obtain additional portraits that would extend the range of crops or minerals, and any suggestions from those with first hand experience with deficiencies will be appreciated.

General Botany.—We have received from Barnes and Noble Inc., 5th Ave. and 18th St., New York, a copy of *An Outline of General Botany* by

HARRY J. FULLER, of the University of Illinois. This is one of the College Outline Series, published by Barnes and Noble, contains 180 pages, 49 text figures, and an index. It retails for 75 cents, with discounts on class supply orders. It offers a rapid survey of the main facts of general botany, and the generalizations which have been achieved in this field. It is a very helpful aid for private study.

WINTHROP JOHN VANLEUVEN OSTERHOUT
AUGUST 2, 1871

THIS NUMBER OF PLANT PHYSIOLOGY

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